Construction of Pbs.PGK.PCRI

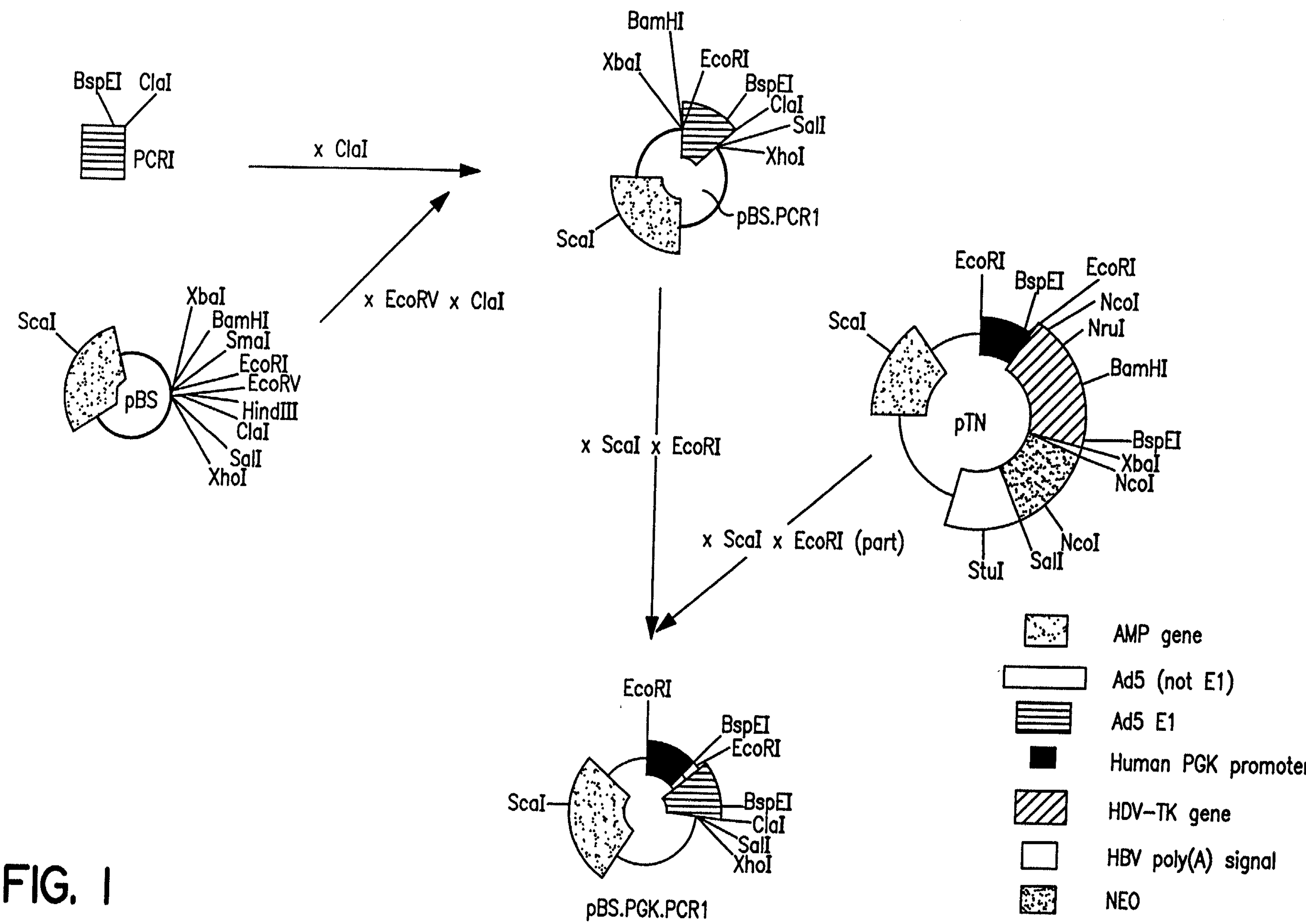


FIG. 1

Construction of pIG.E1a.E1b.X

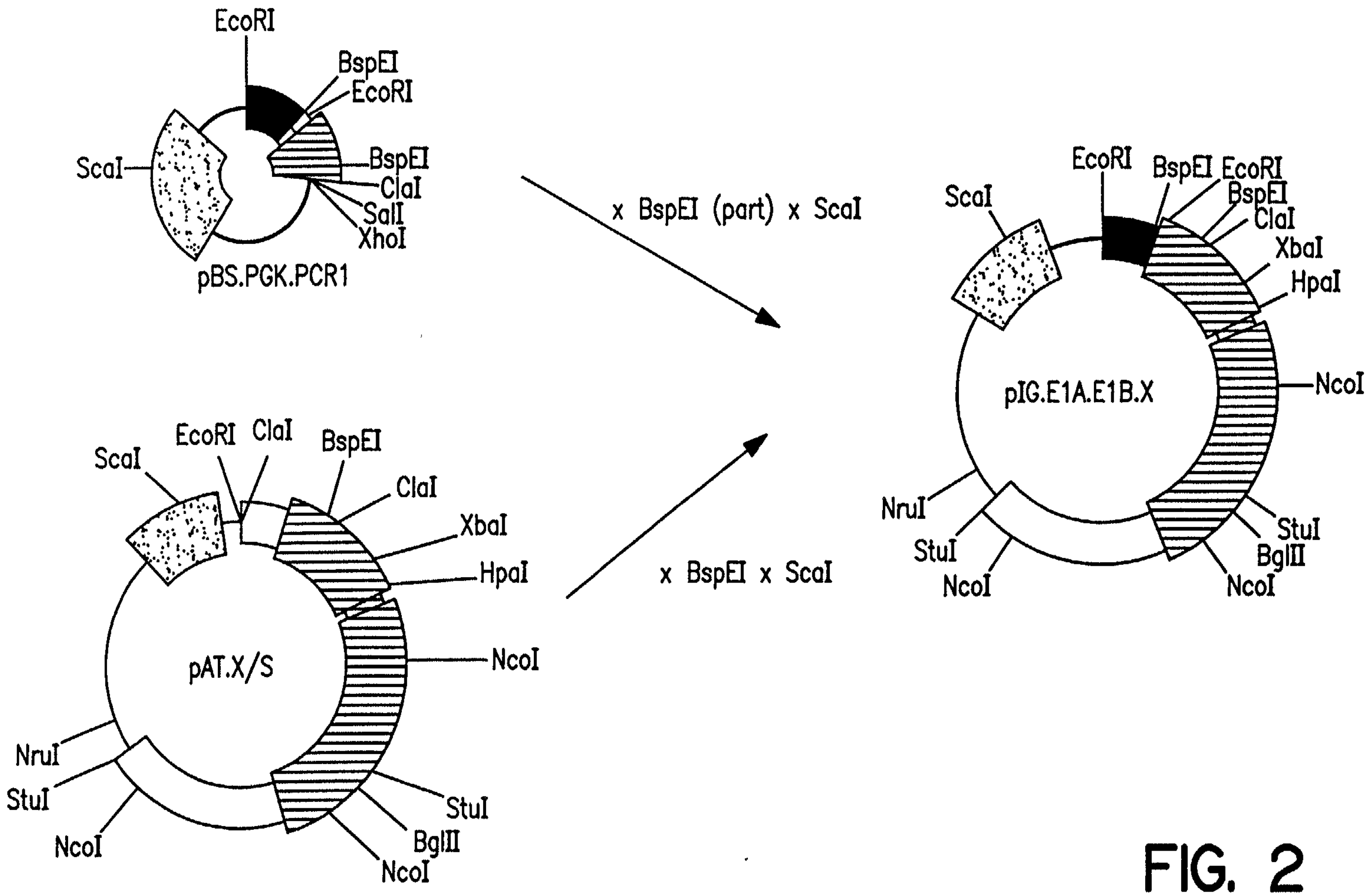


FIG. 2

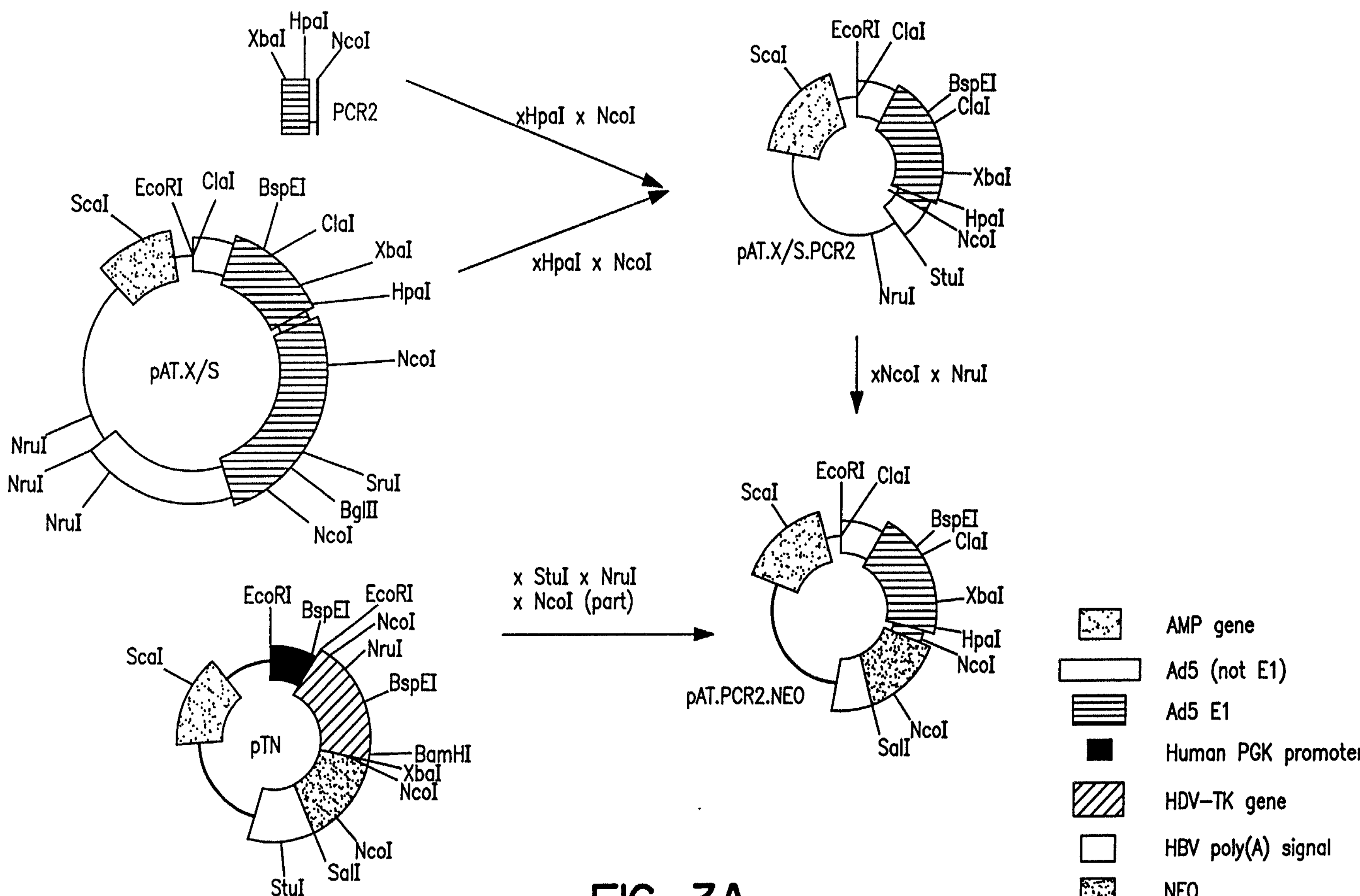

XbaI
HpaI
NcoI
PCR2
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.X/S
NruI
NruI
NruI
SruI
BglII
NcoI
xHpaI x NcoI
xHpaI x NcoI
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.X/S.PCR2
StuI
NruI
xNcoI x NruI
EcoRI
BspEI
EcoRI
NcoI
NruI
ScaI
BspEI
pTN
BamHI
XbaI
NcoI
NcoI
SalI
StuI
x StuI x NruI
x NcoI (part)
ScaI
EcoRI
ClaI
BspEI
ClaI
XbaI
HpaI
NcoI
pAT.PCR2.NEO
NcoI
SalI
AMP gene
Ad5 (not E1)
Ad5 E1
Human PGK promoter
HDV-TK gene
HBV poly(A) signal
NEO

FIG. 3A

Construction of pIG.E1a.NEO

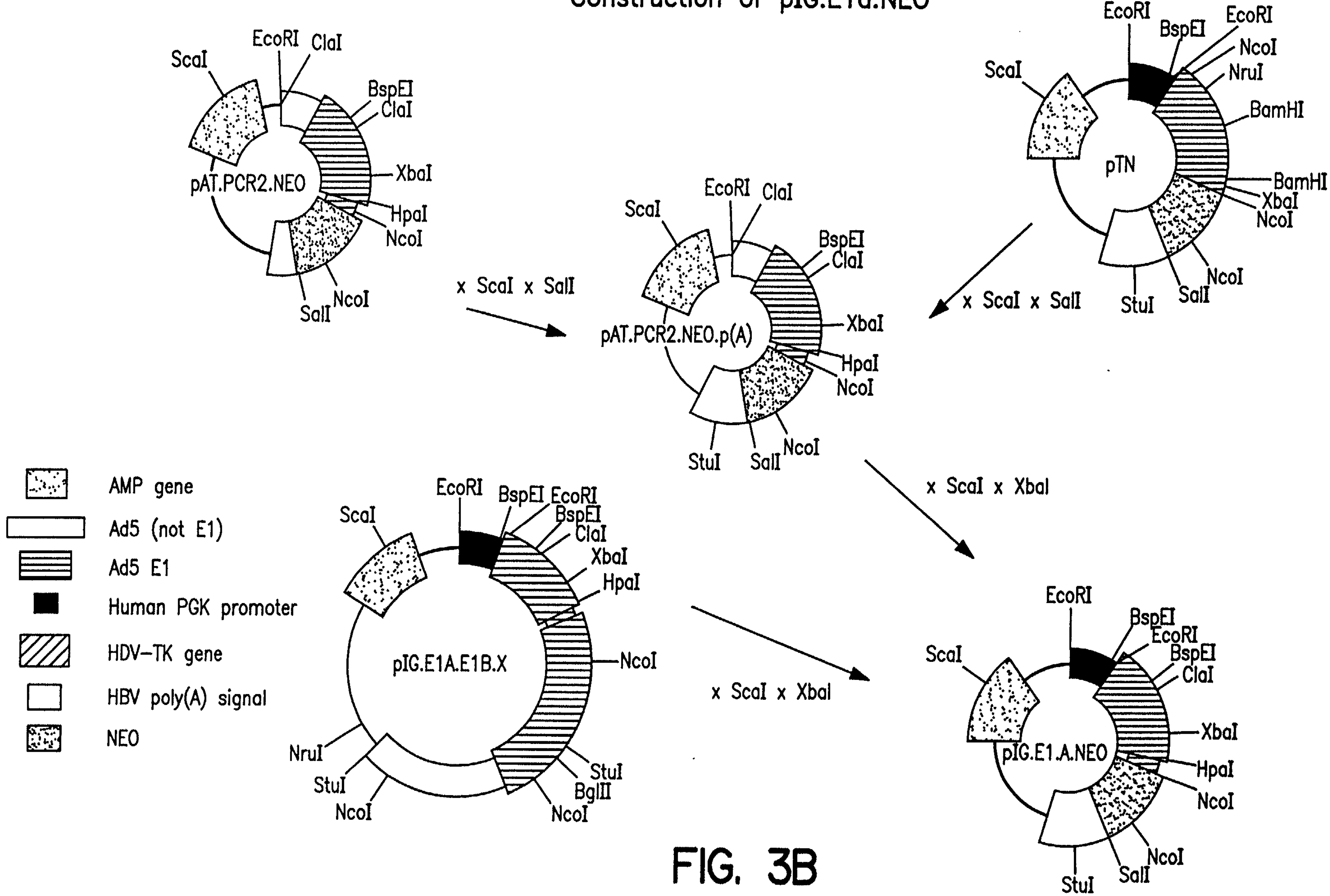
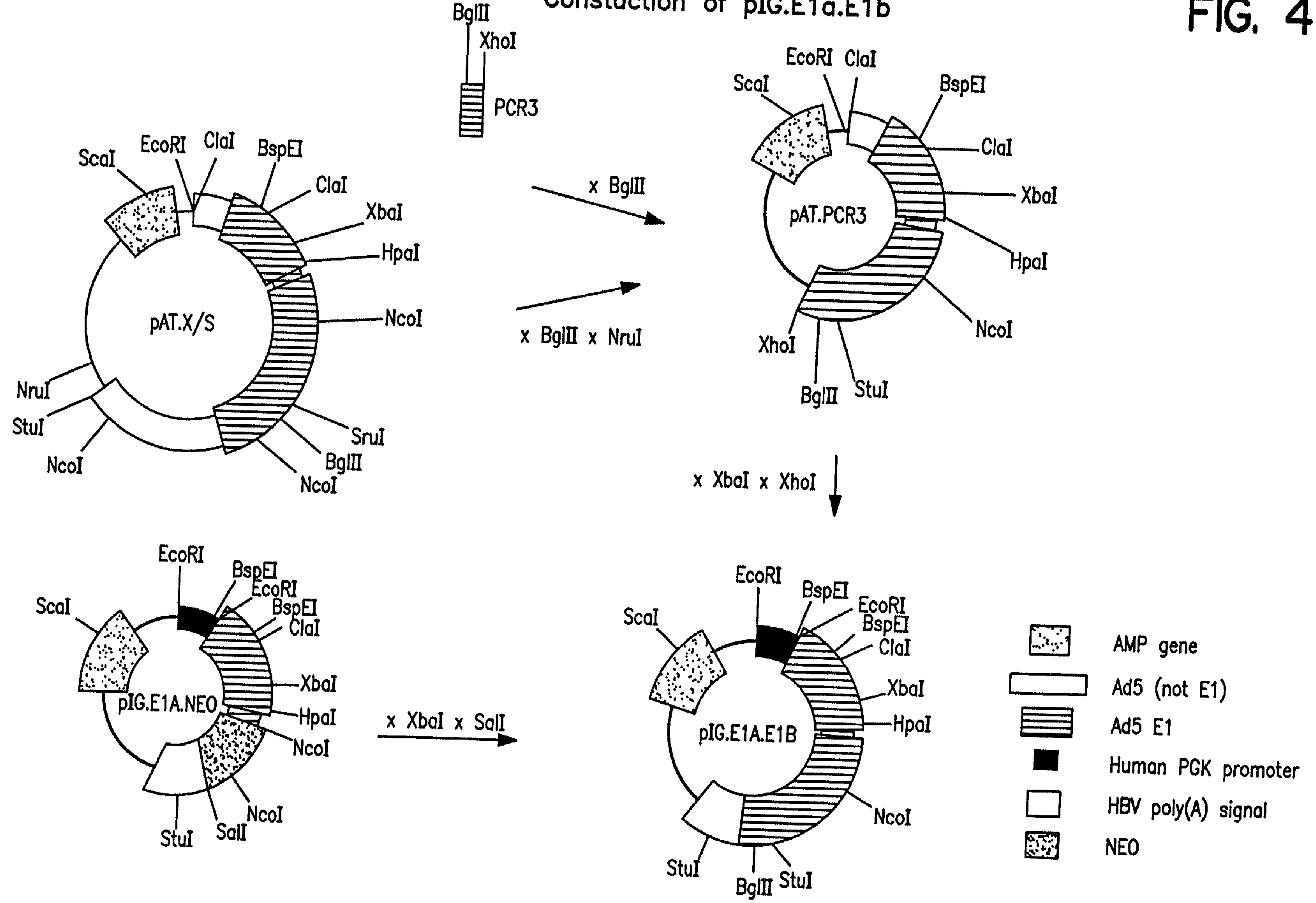


FIG. 3B

Constuction of pIG.E1a.E1b

FIG. 4



Construction of pIG.NEO

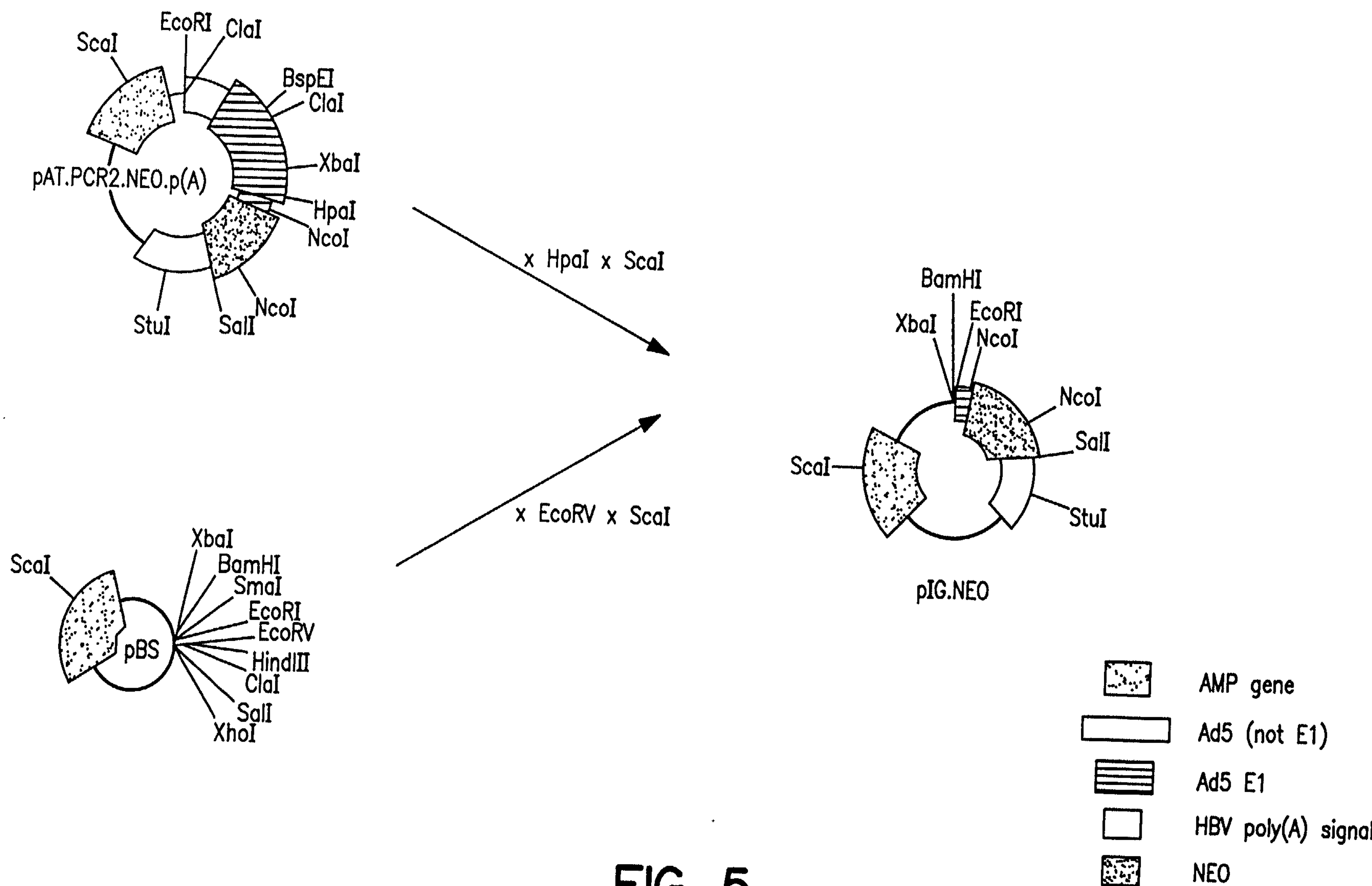
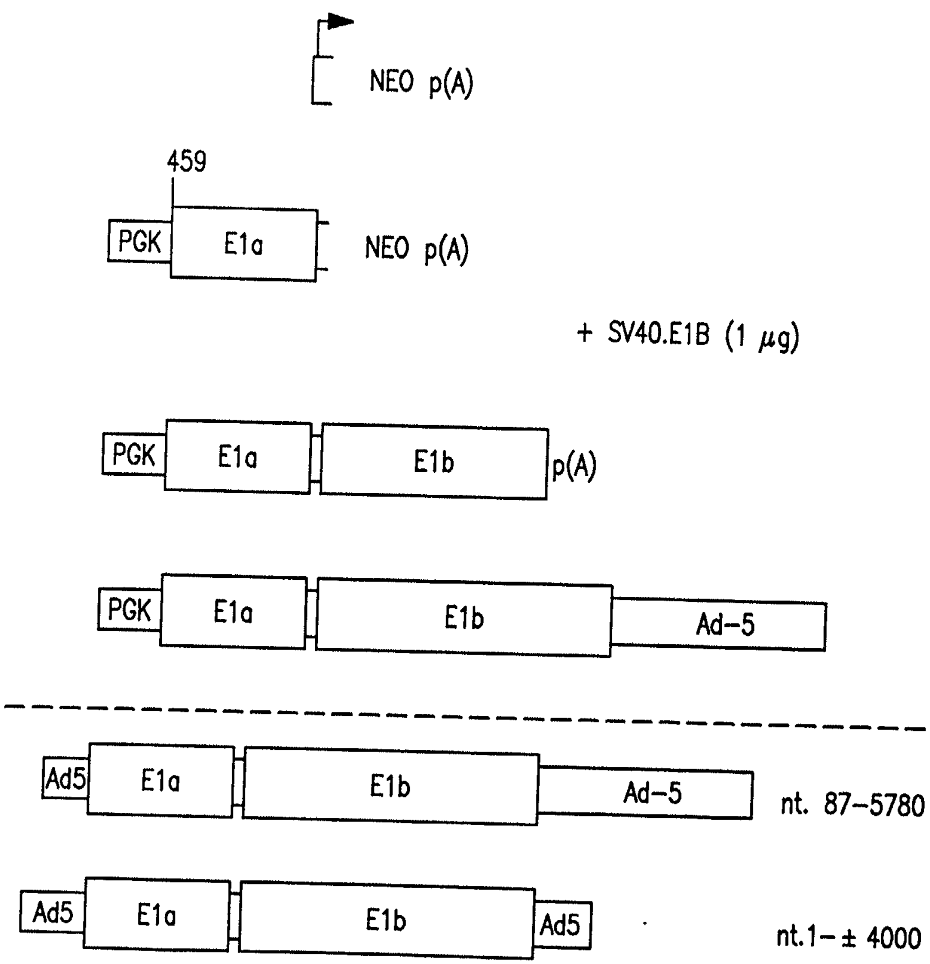


FIG. 5

Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



	transformation of primary kidney cells 1μg	5μg
pIG.NEO	nd	nd
pIG.E1a.NEO	1	nd
+ SV40.E1B (1 μg)	18	nd
pIG.E1a.E1b	8	57
pIG.E1a.E1b X	10	27
911 cells	13	nd
293 cells	nd	nd

*average of 5 plates 21 days after transelection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

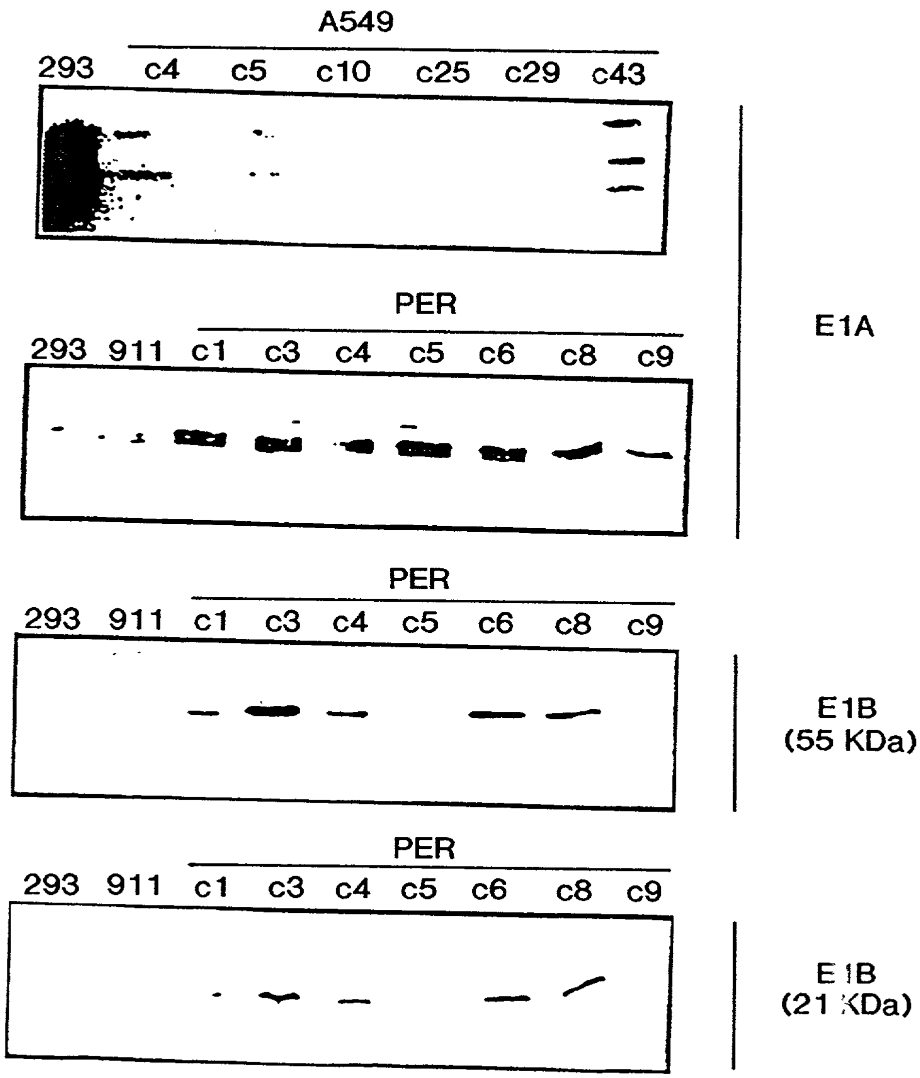


FIG. 7

Southern blot analyses of 293, 911 and PER cell lines

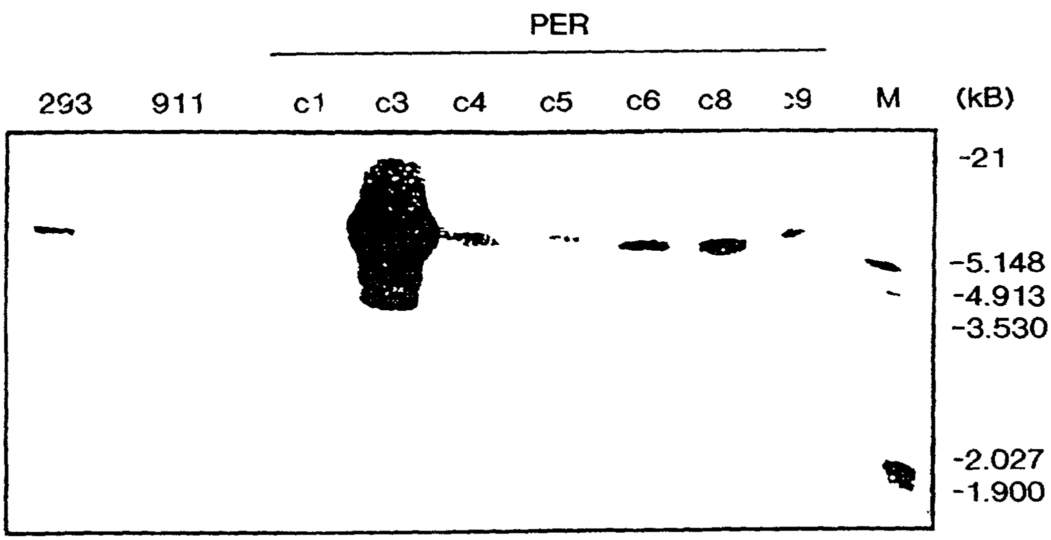


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5 μg pRSV.lasZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

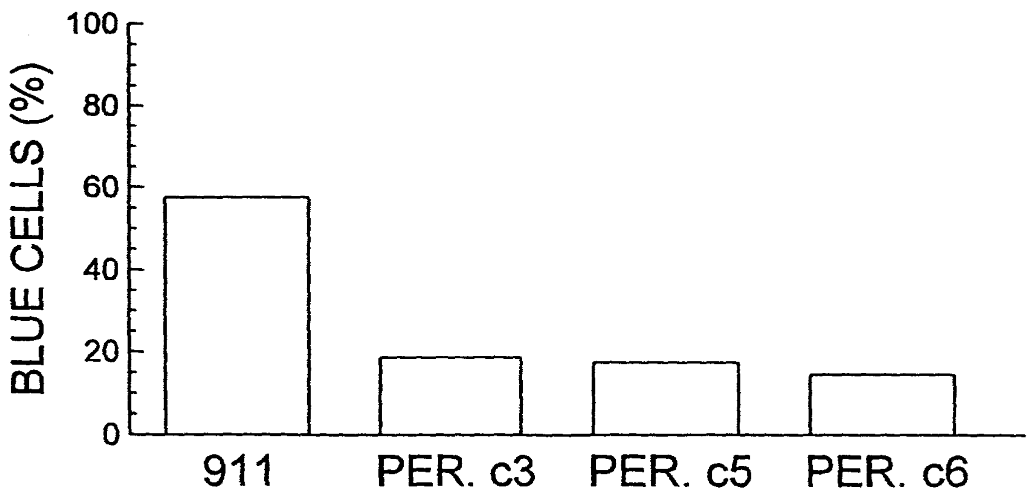


FIG. 9

Construction of pMLP1.TK

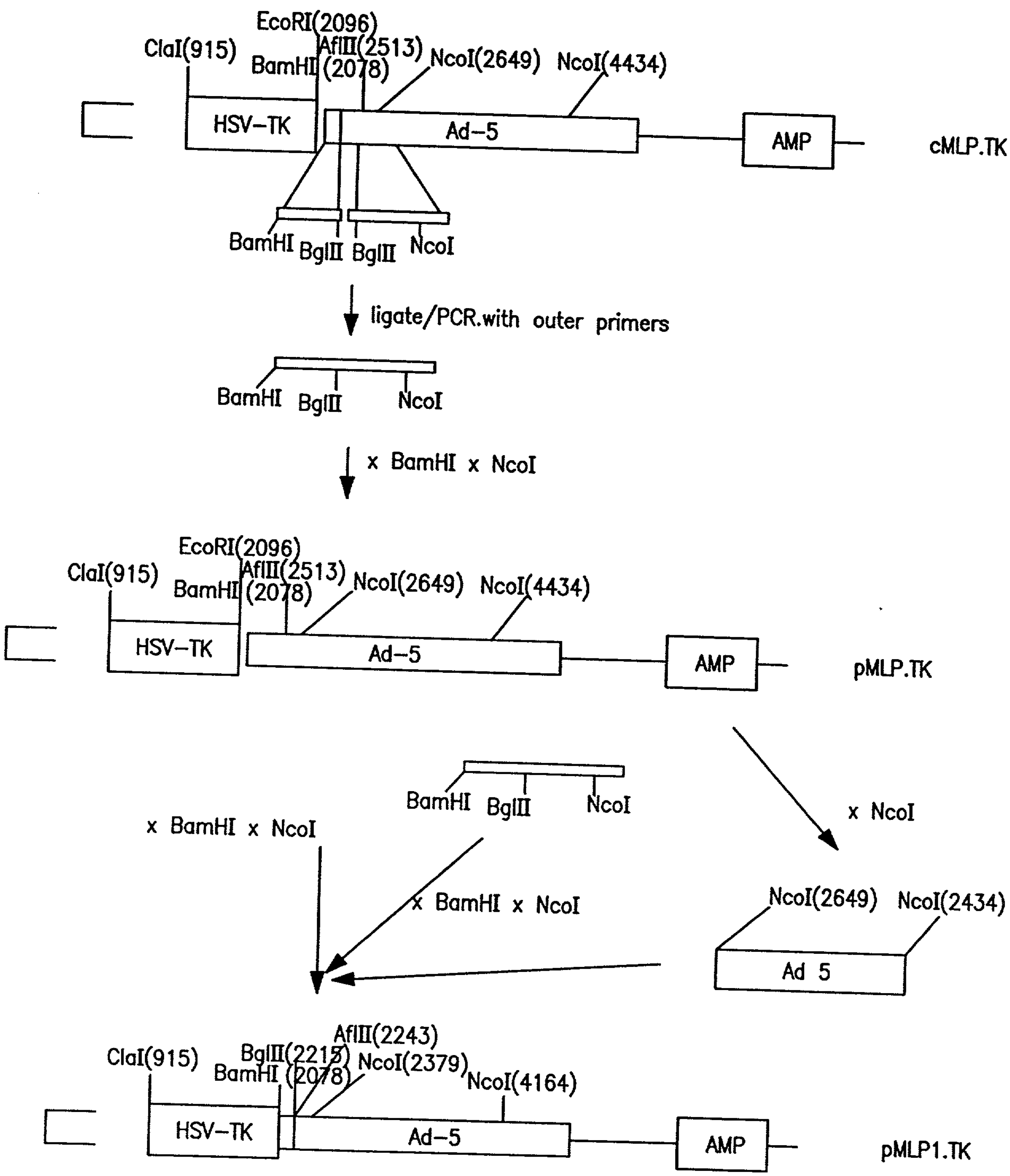
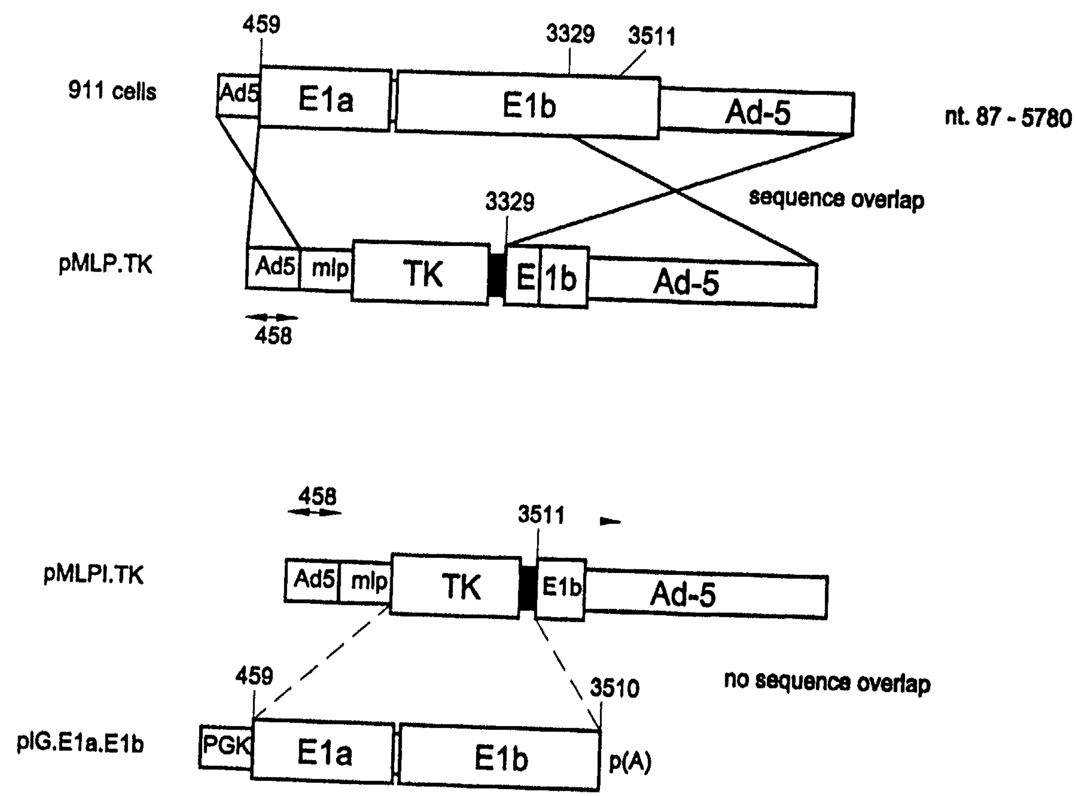


FIG. 10

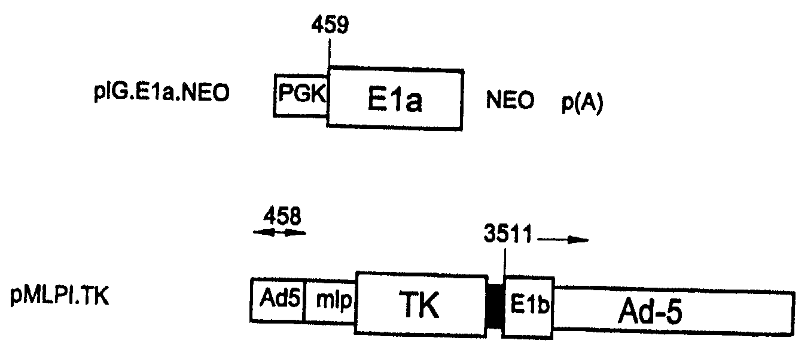
New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on primary cells

FIG. 11A

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418

FIG. I IB

Generation of recombinant adenovirus

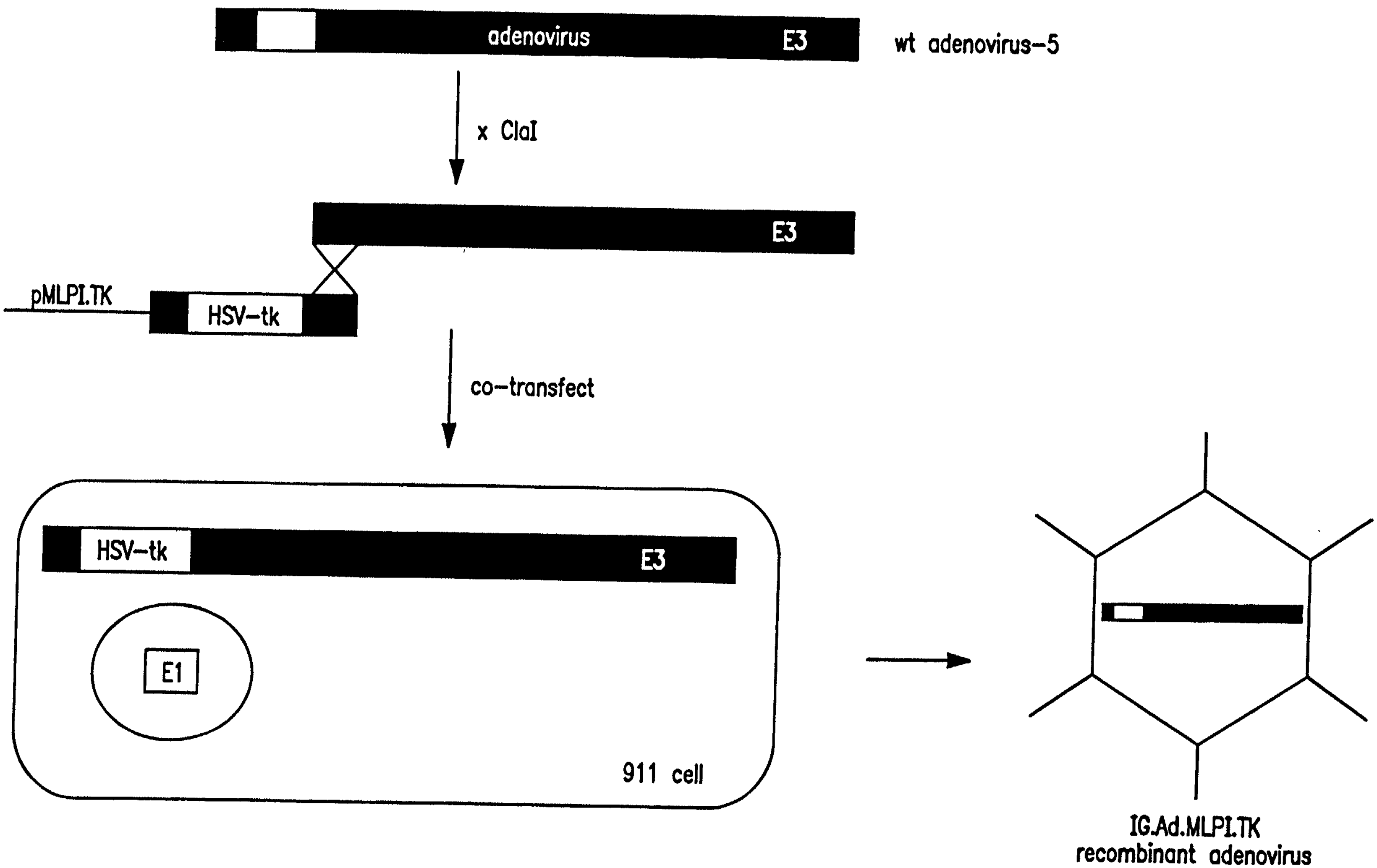


FIG. 12

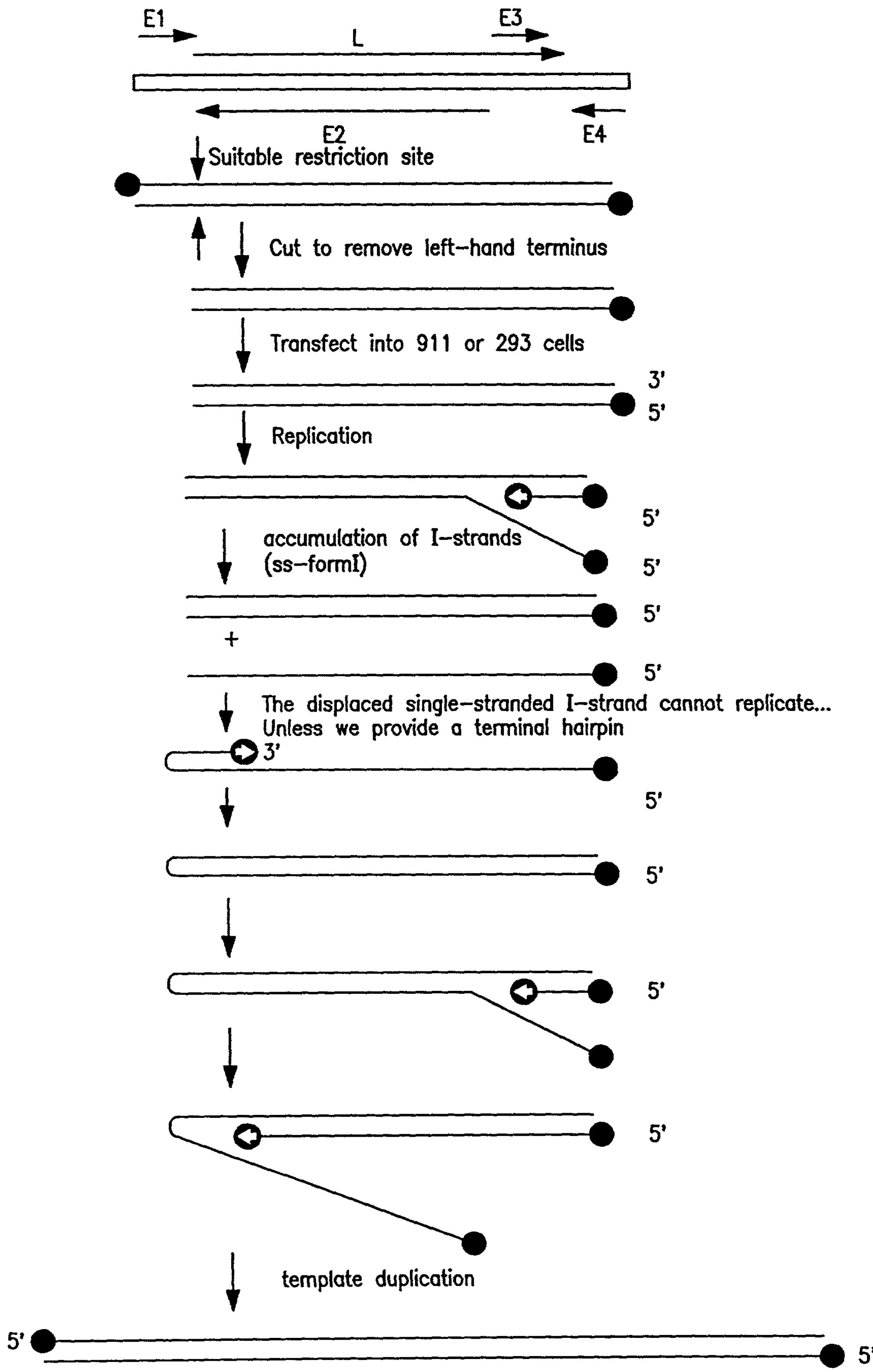

E1
E3
L
E2
E4
Suitable restriction site
Cut to remove left-hand terminus
Transfect into 911 or 293 cells
3'
5'
Replication
5'
accumulation of I-strands
(ss-formI)
5'
5'
+
5'
The displaced single-stranded I-strand cannot replicate...
Unless we provide a terminal hairpin
3'
5'
5'
5'
5'
template duplication
5'
5'

FIG. 13

Replication of Adenovirus

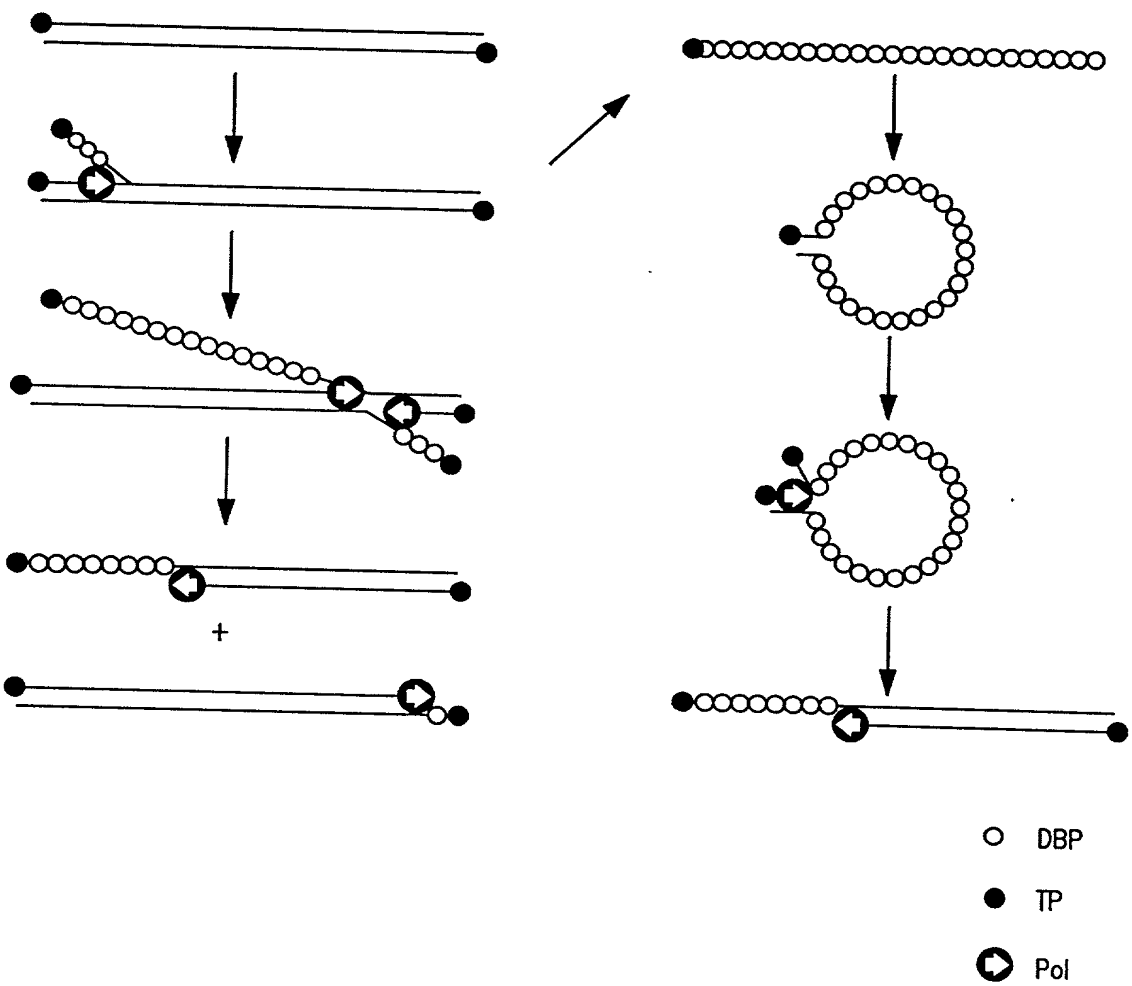


FIG. 14

The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases ***Asp718I*** of plasid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.


```
5'-GTACACTGACCTAGTGCCGCCCGGGCA
             ||||||||||||||||| A
          3'-GATCACGGCGGGCCCGA
```

FIG. 15

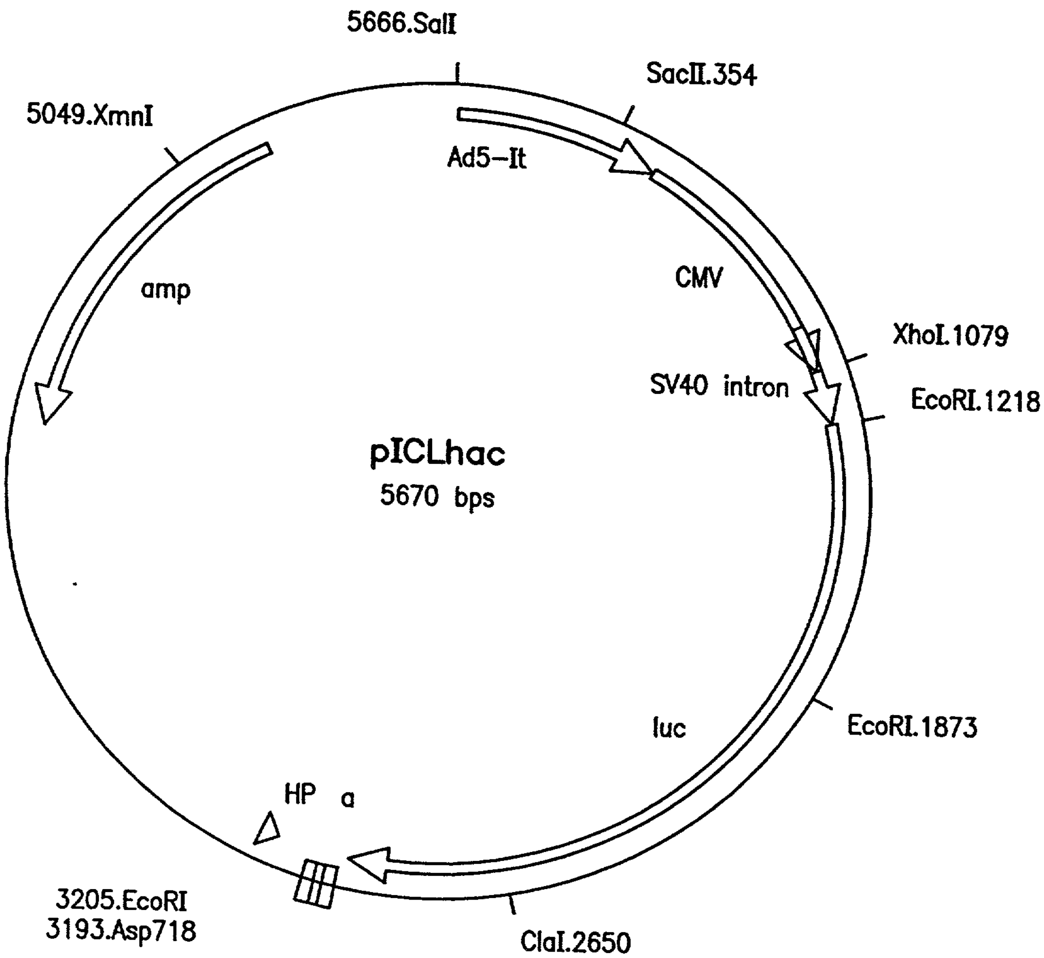

5666.SalI
SacII.354
5049.XmnI
Ad5-It
CMV
amp
XhoI.1079
SV40 intron
EcoRI.1218
pICLhac
5670 bps
luc
EcoRI.1873
HP a
3205.EcoRI
3193.Asp718
ClaI.2650

FIG. 16

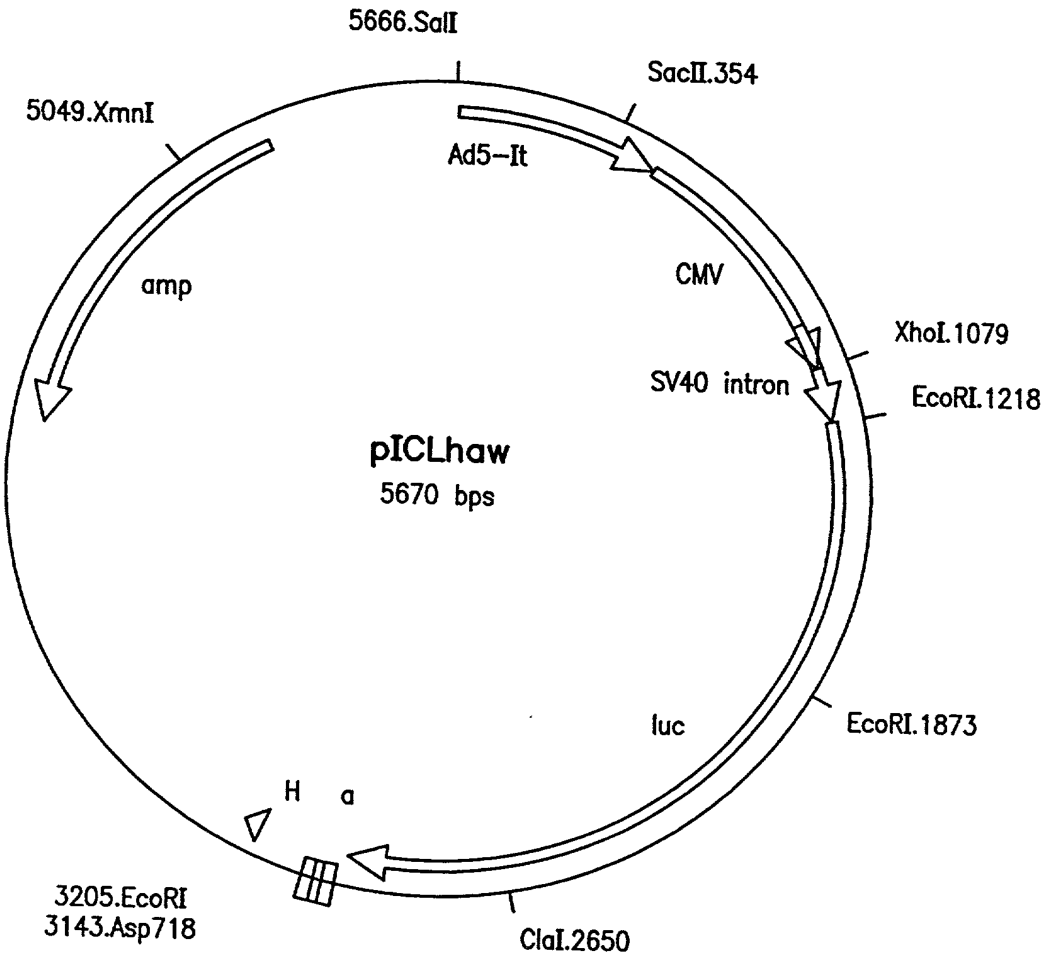

5666.SalI
SacII.354
5049.XmnI
Ad5-It
CMV
amp
XhoI.1079
SV40 intron
EcoRI.1218
pICLhaw
5670 bps
luc
EcoRI.1873
H
a
3205.EcoRI
3143.Asp718
ClaI.2650

FIG. 17

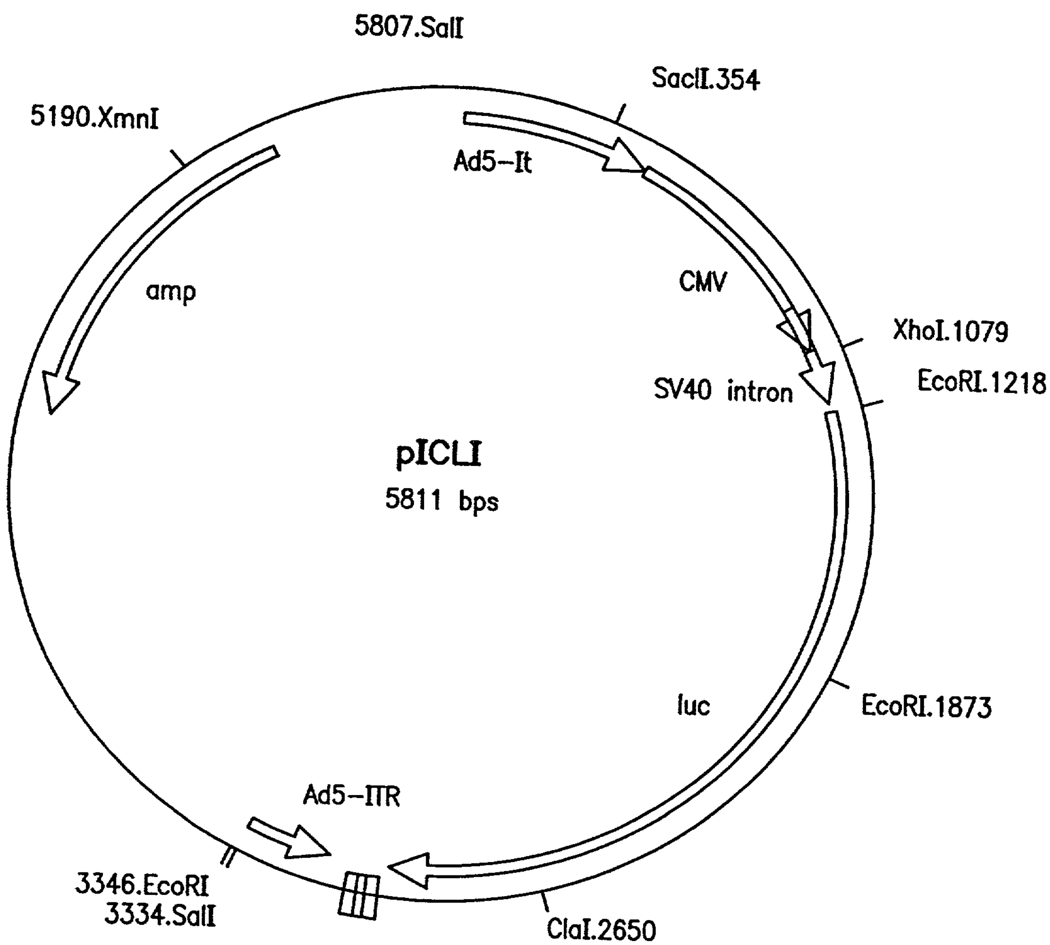

5807.SalI
SacII.354
5190.XmnI
Ad5-It
amp
CMV
XhoI.1079
SV40 intron
EcoRI.1218
pICLI
5811 bps
luc
EcoRI.1873
Ad5-ITR
3346.EcoRI
3334.SalI
ClaI.2650

FIG. 18

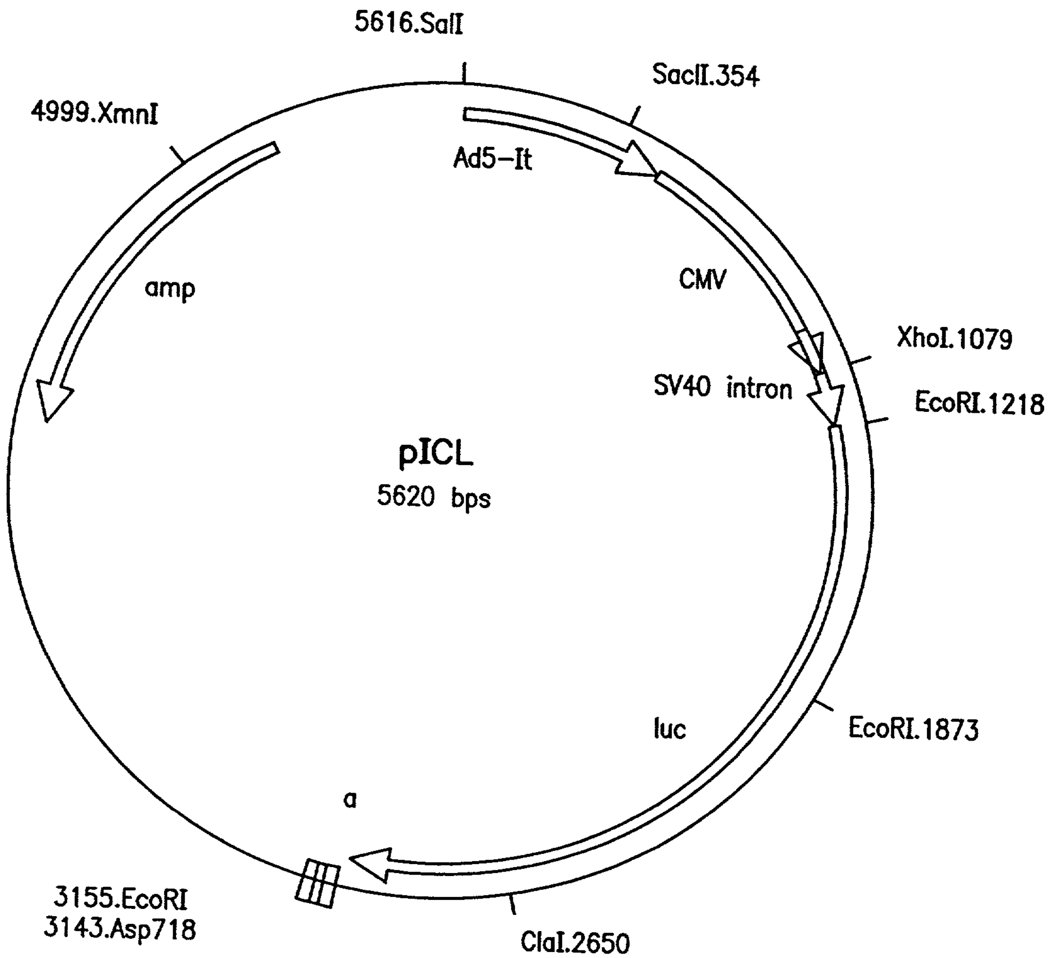

5616.SalI
SacII.354
4999.XmnI
Ad5-It
amp
CMV
XhoI.1079
SV40 intron
EcoRI.1218
pICL
5620 bps
luc
EcoRI.1873
a
3155.EcoRI
3143.Asp718
ClaI.2650

FIG. 19

Cloned adenovirous fragments

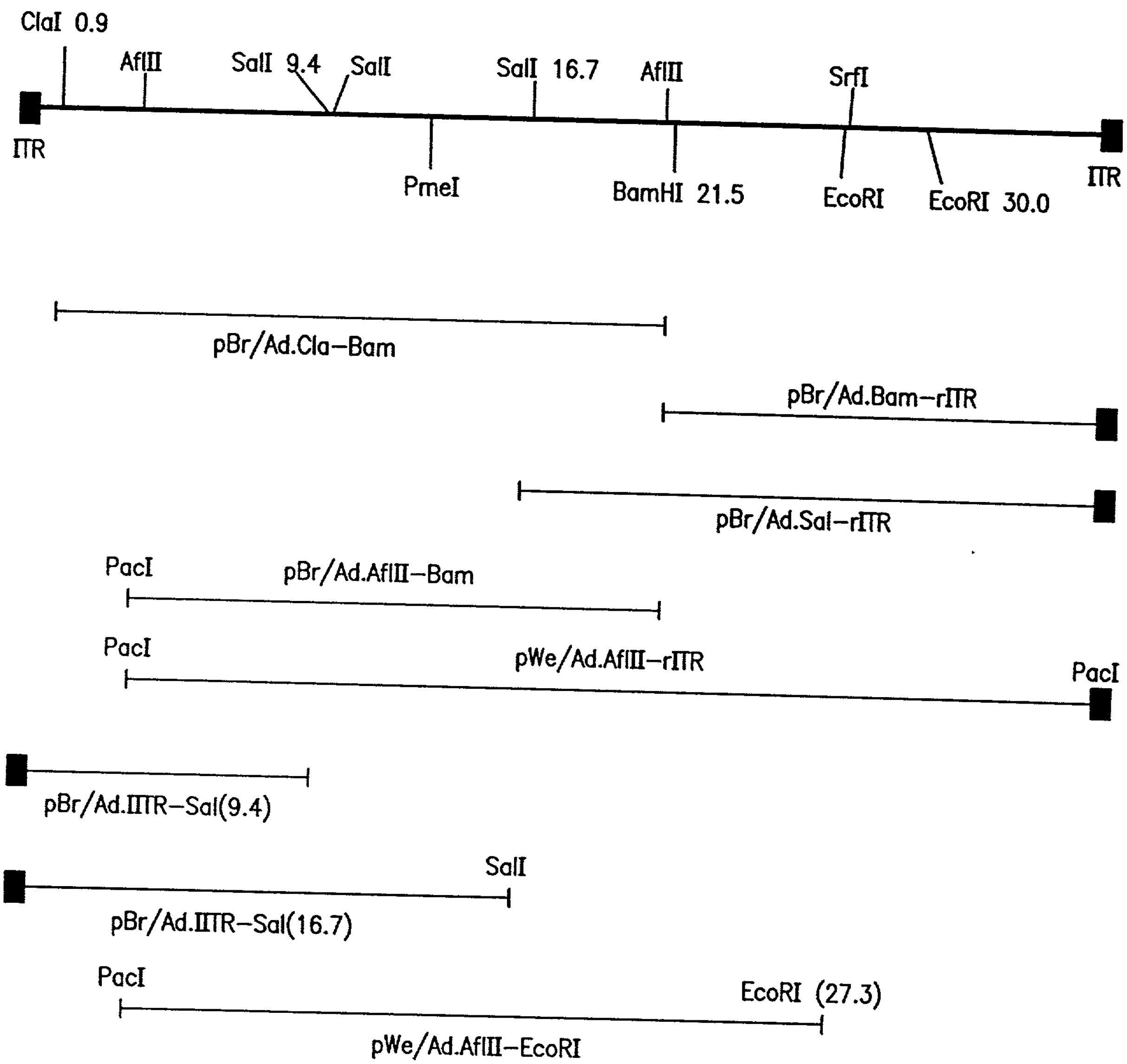


FIG. 20

Adapter plasmid pAd5/L420-HSA

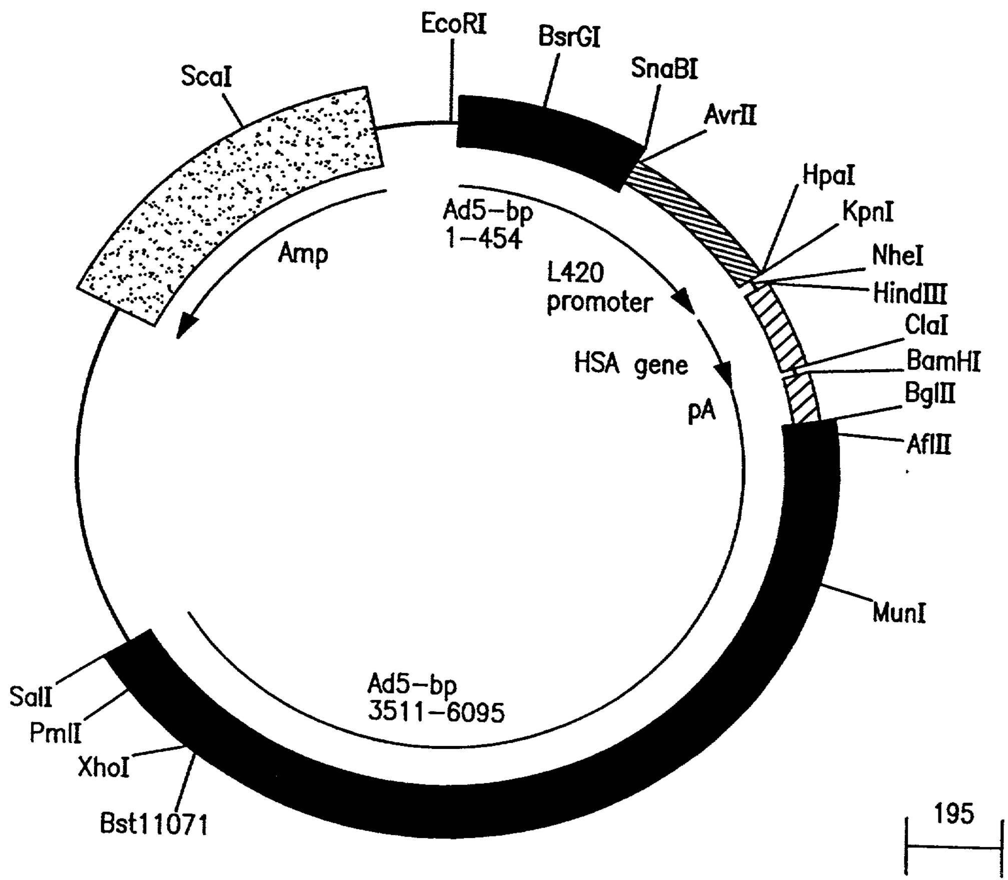


FIG. 21

Adapter plasmid pAd5/CLIP

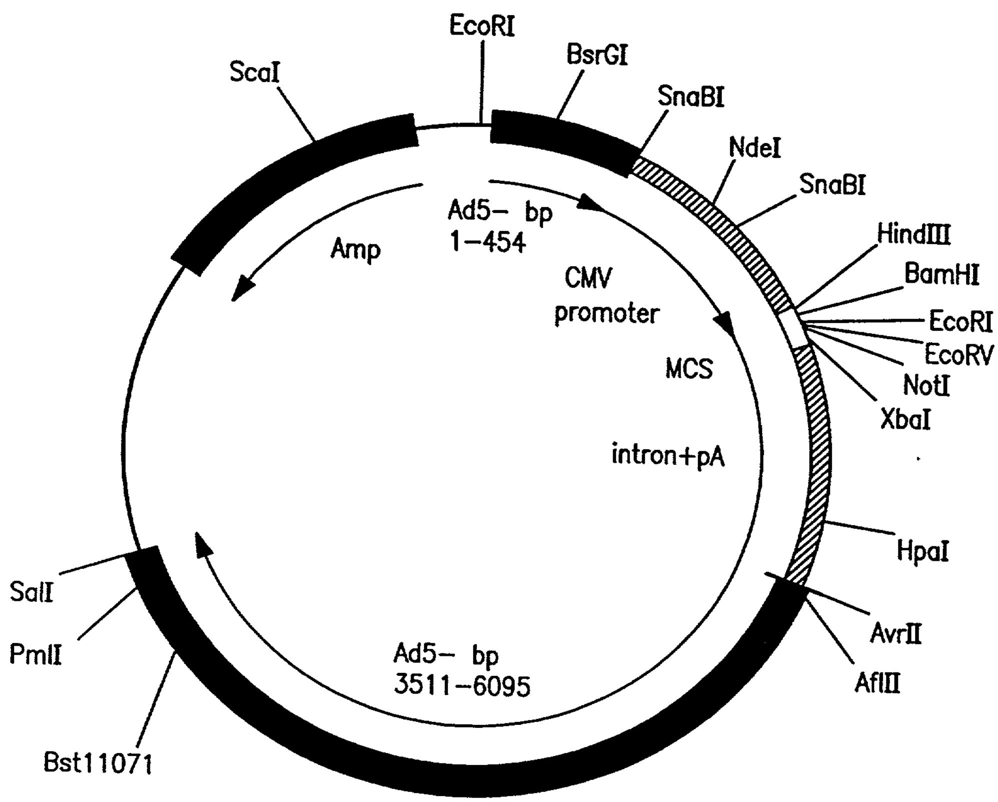


FIG. 22

Generation of recombinant adenoviruses

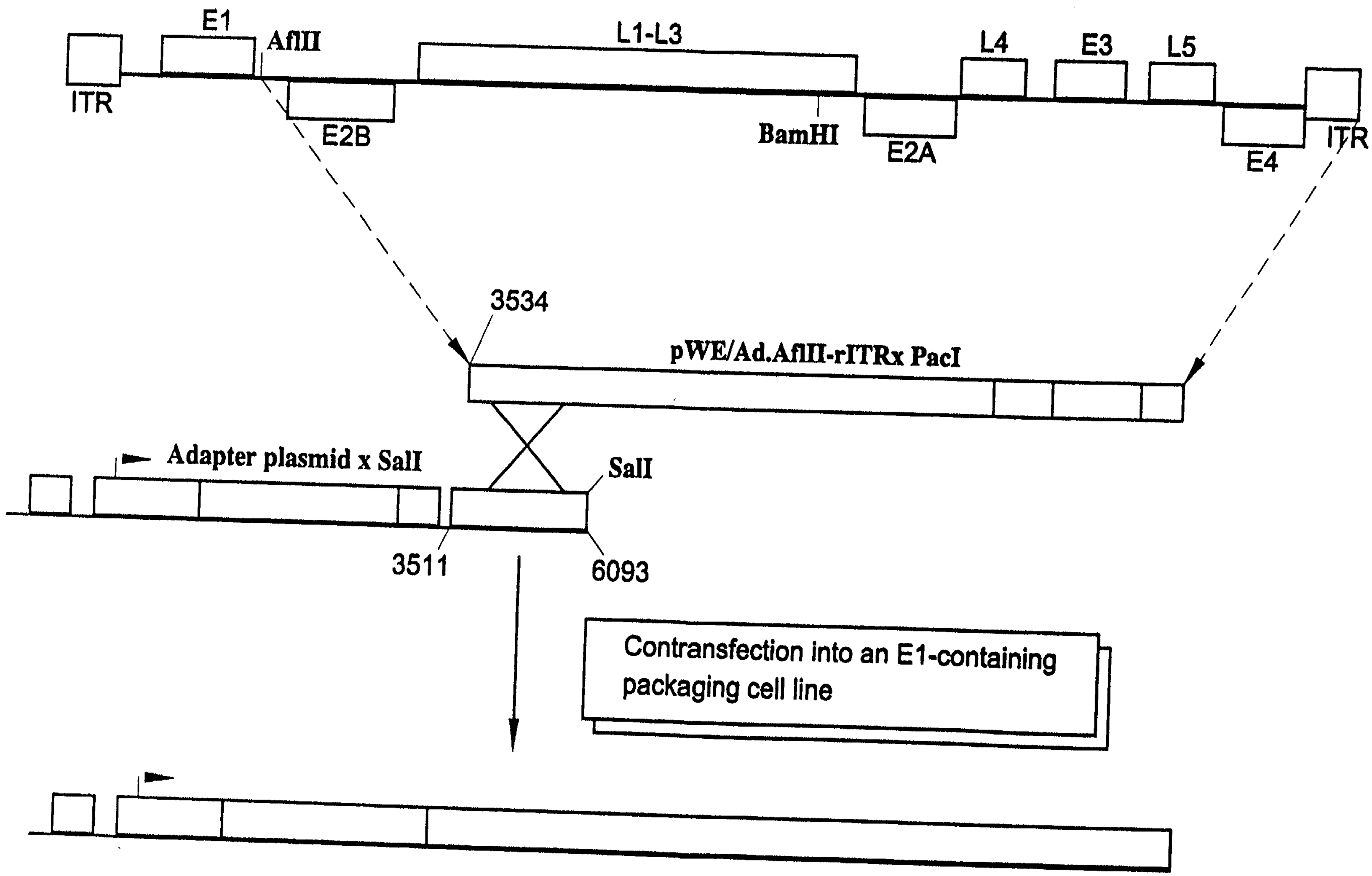


FIG. 23

Minimal adenovirus vector pMV/L420H

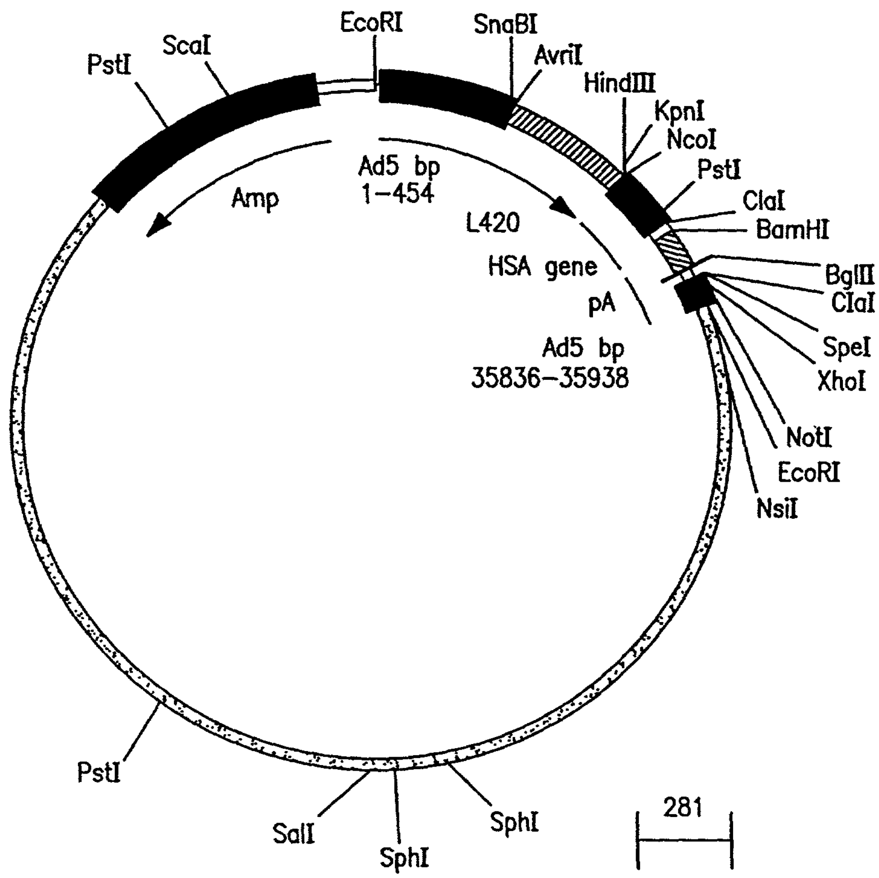


FIG. 24

Construction of pWE/AdΔ5'

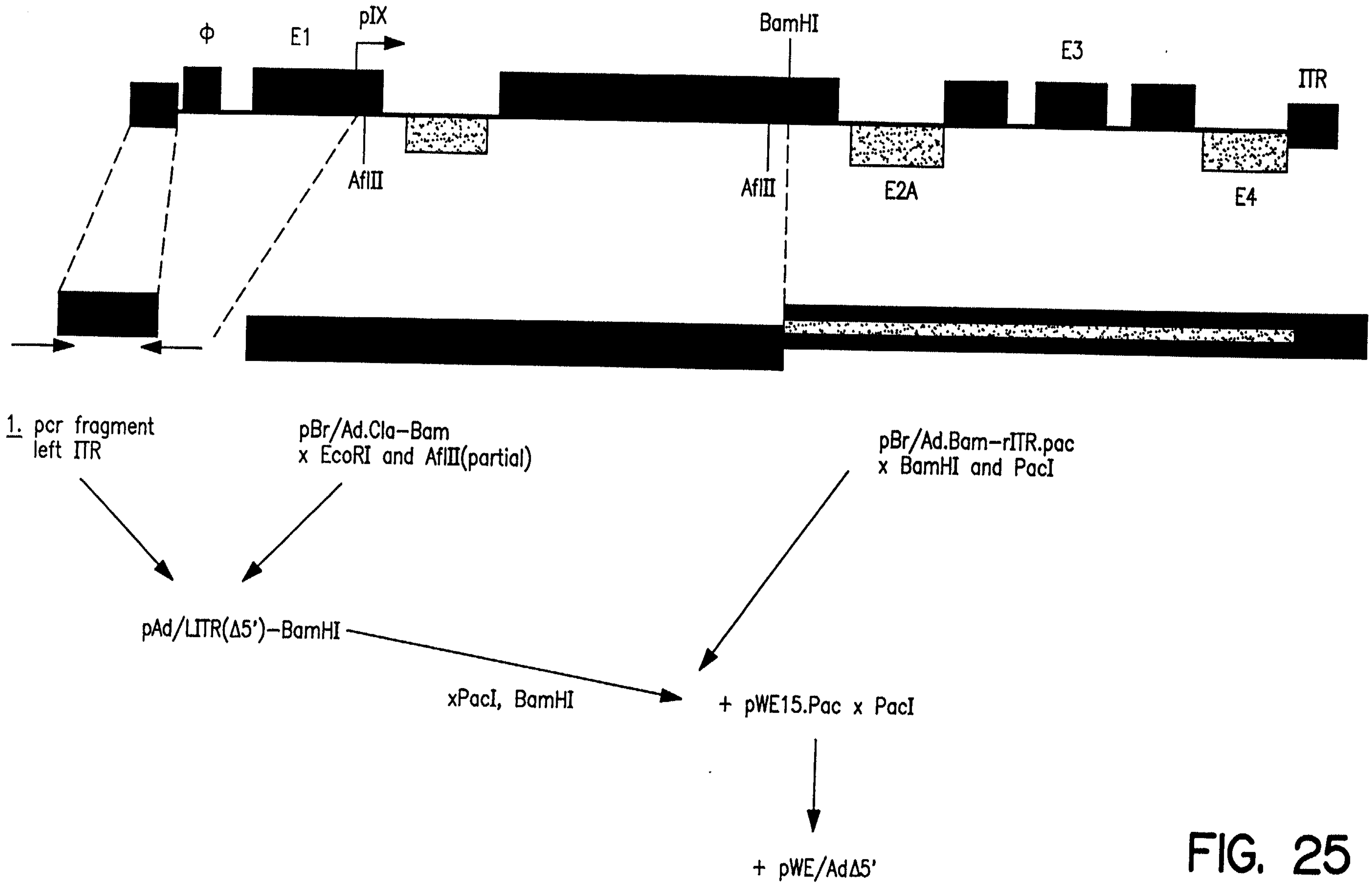


FIG. 25

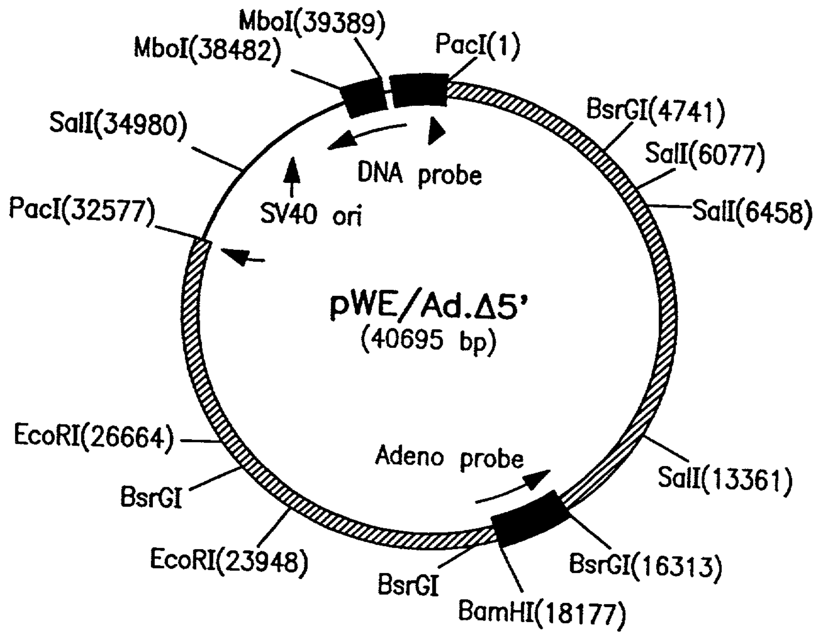

MboI(39389)
MboI(38482)
PacI(1)
BsrGI(4741)
SalI(34980)
SalI(6077)
DNA probe
SalI(6458)
PacI(32577)
SV40 ori
pWE/Ad.Δ5'
(40695 bp)
EcoRI(26664)
Adeno probe
SalI(13361)
BsrGI
EcoRI(23948)
BsrGI(16313)
BsrGI
BamHI(18177)

FIG. 26A

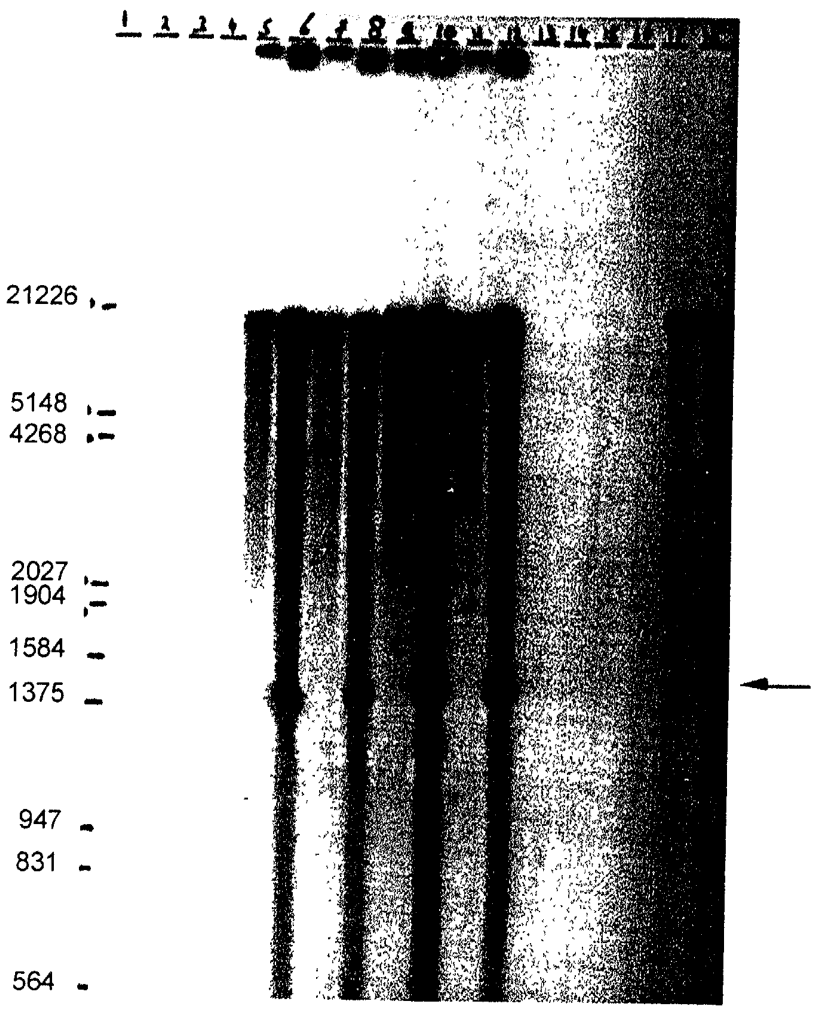


FIG. 26B

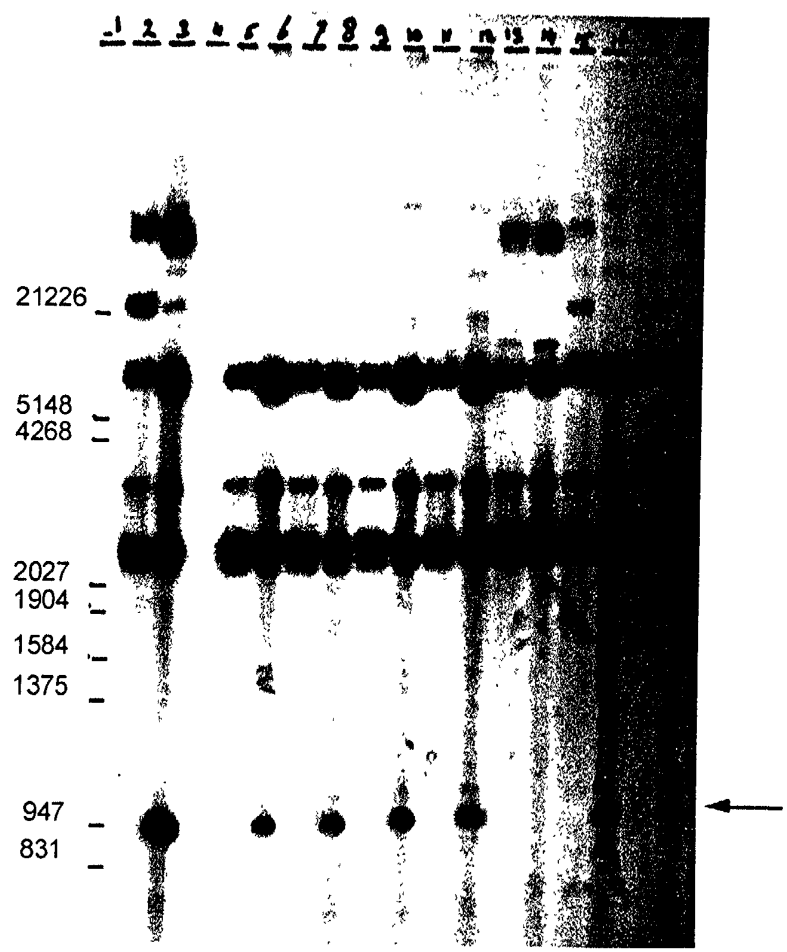
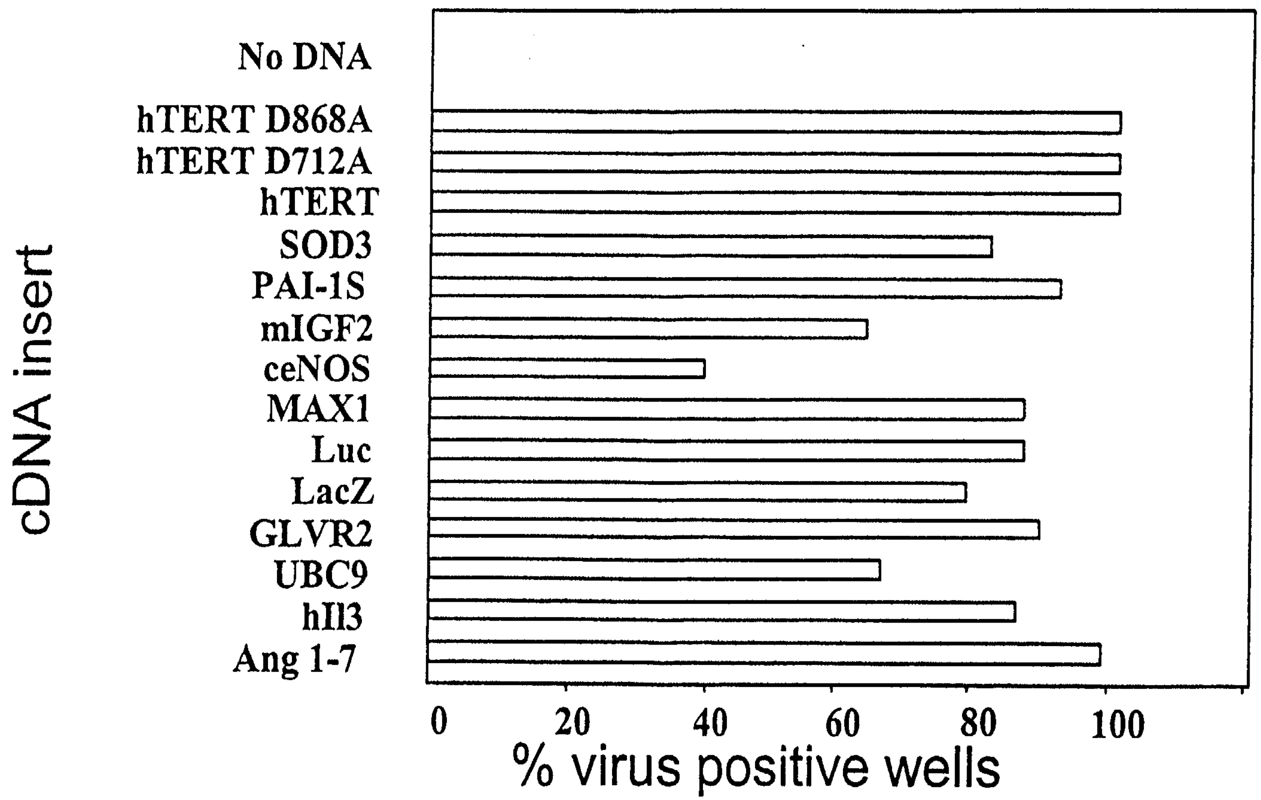


FIG. 26C



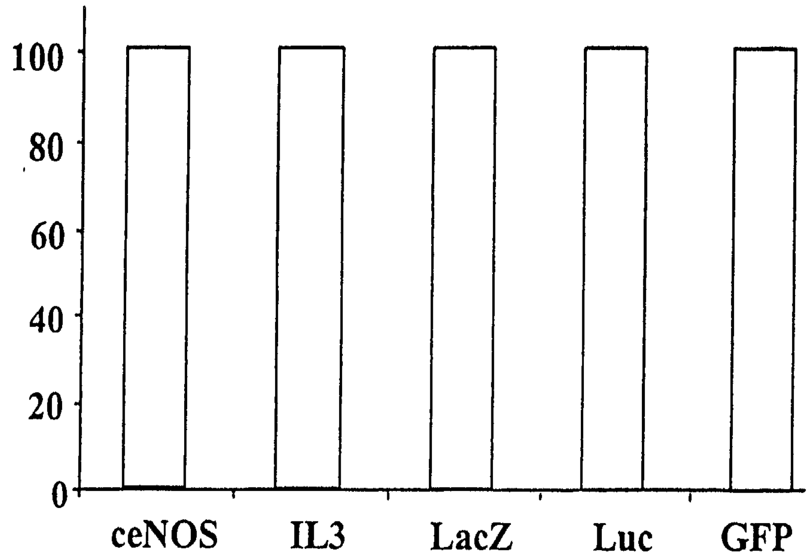
Average percentage CPE efficiency: 86 %

FIG. 27

Gene	Insert kb
• ceNOS	3.6
• hTERT	3.5
• hTERT D712A	3.5
• lacZ	3.2
• hCAT1	2.2
• GLVR2	2.0
• Luc	1.7
• SOD3	1.4
• MAX1	.550
• hVEGF121	.511
• hIL3	.434
• UBC9	.412
• ANG1-7	.104

Average titer
$0.8 \pm 0.7 \times 10^9$ pfu/ml

FIG. 28



Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29

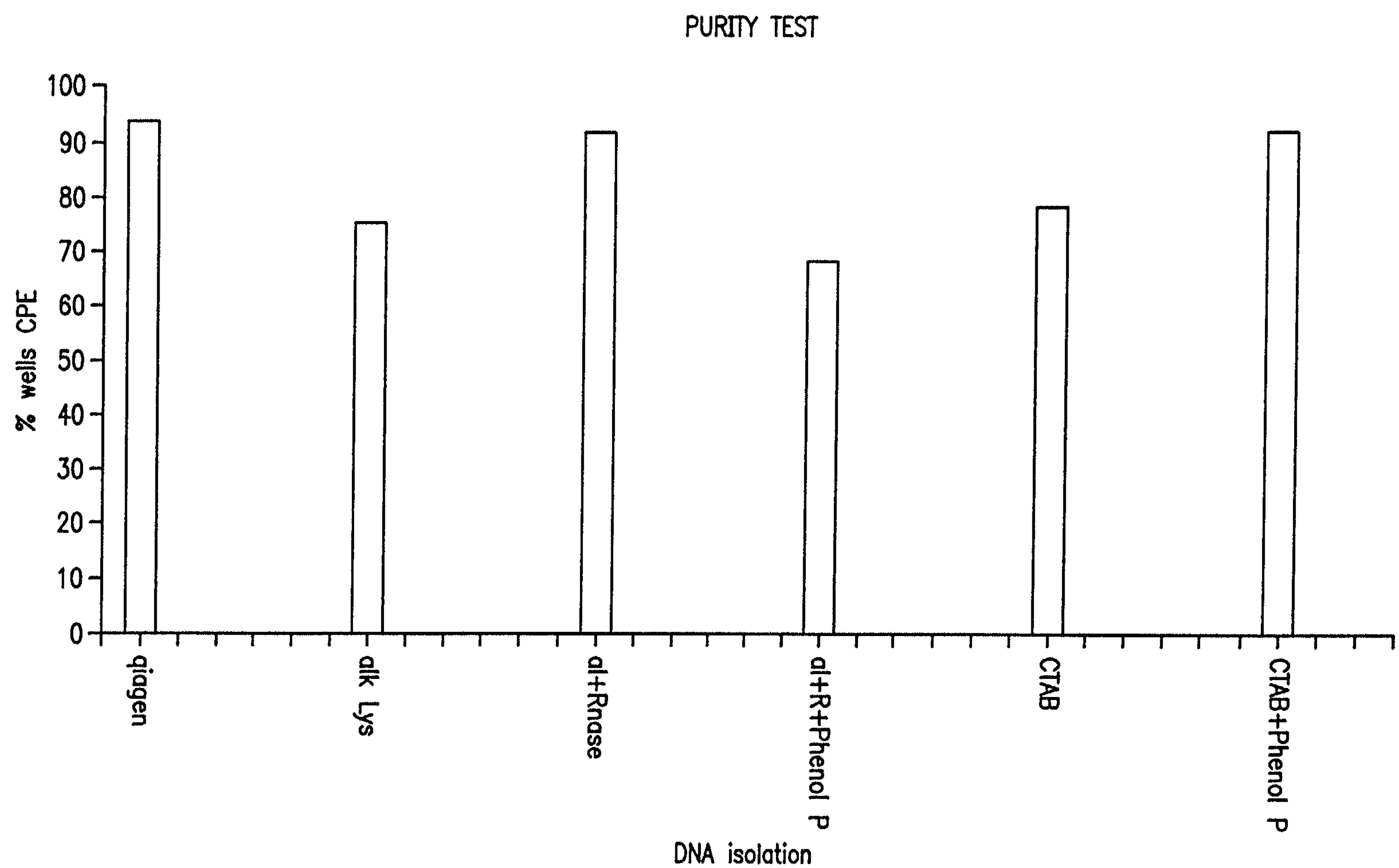

PURITY TEST
% wells CPE
100
90
80
70
60
50
40
30
20
10
0
qiagen
alk Lys
al+Rnase
al+R+Phenol P
CTAB
CTAB+Phenol P
DNA isolation

FIG. 30

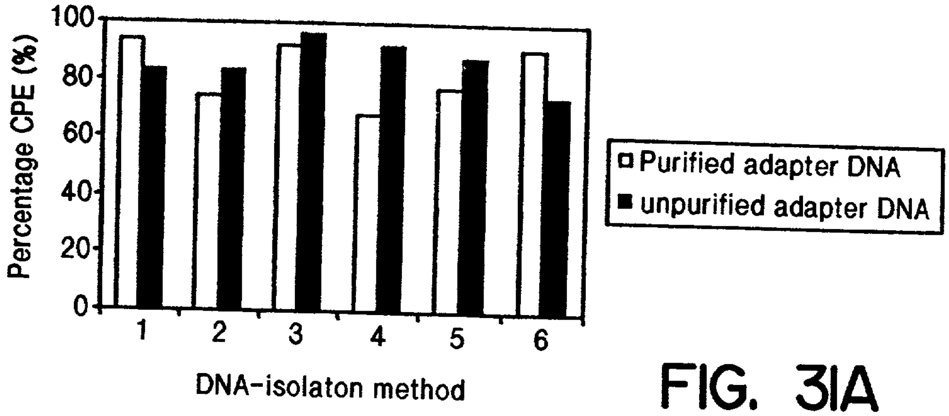


FIG. 31A

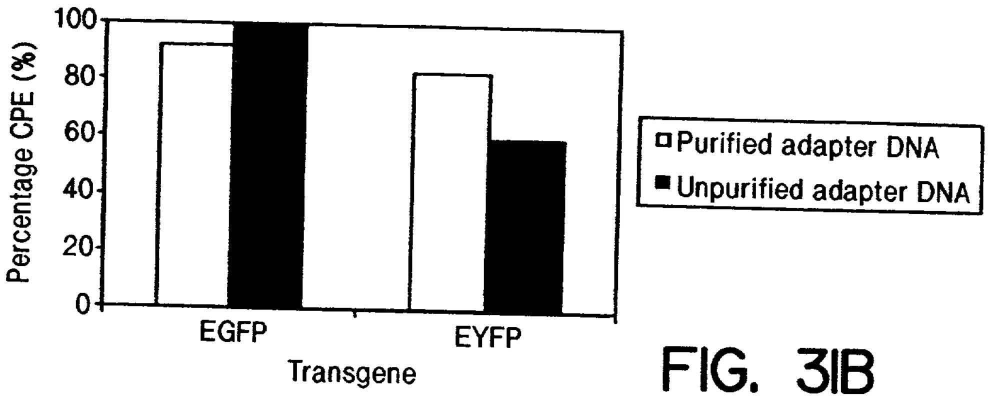


FIG. 31B

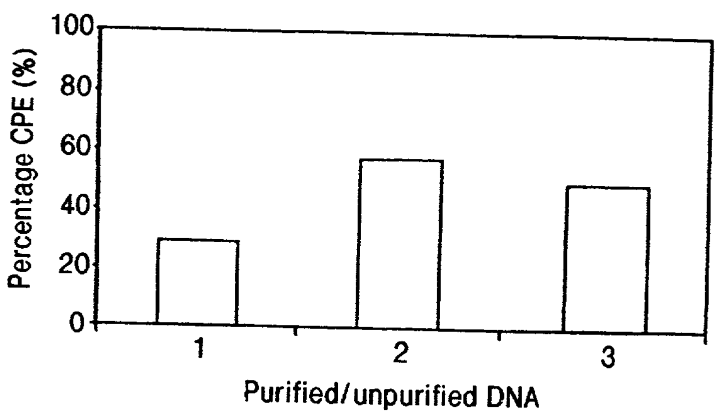


FIG. 31C

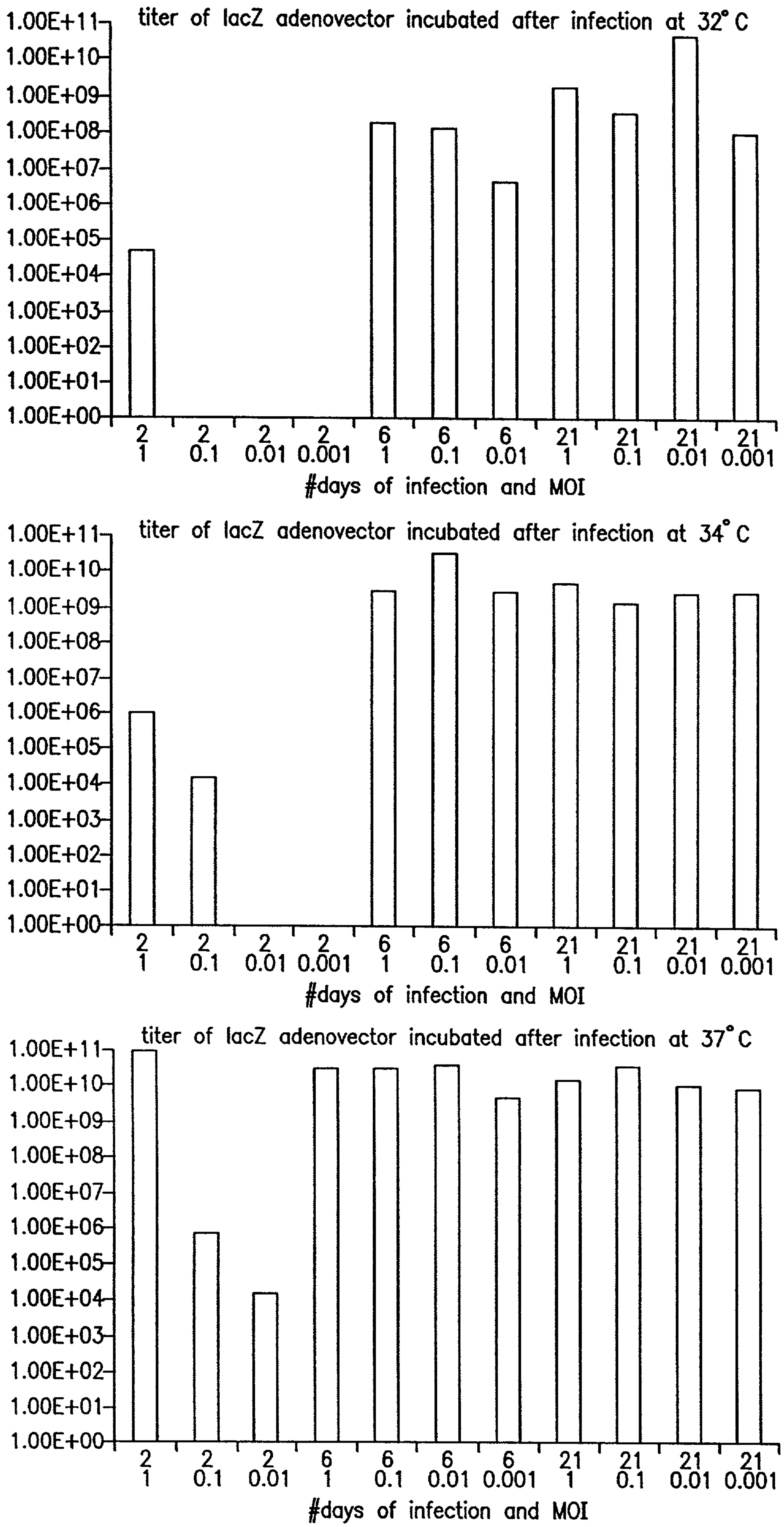

titer of lacZ adenovector incubated after infection at 32° C
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
2 1
2 0.1
2 0.01
2 0.001
6 1
6 0.1
6 0.01
21 1
21 0.1
21 0.01
21 0.001
#days of infection and MOI
titer of lacZ adenovector incubated after infection at 34° C
#days of infection and MOI
titer of lacZ adenovector incubated after infection at 37° C
2 1
2 0.1
2 0.01
6 1
6 0.1
6 0.01
6 0.001
21 1
21 0.1
21 0.01
21 0.001
#days of infection and MOI

FIG. 32

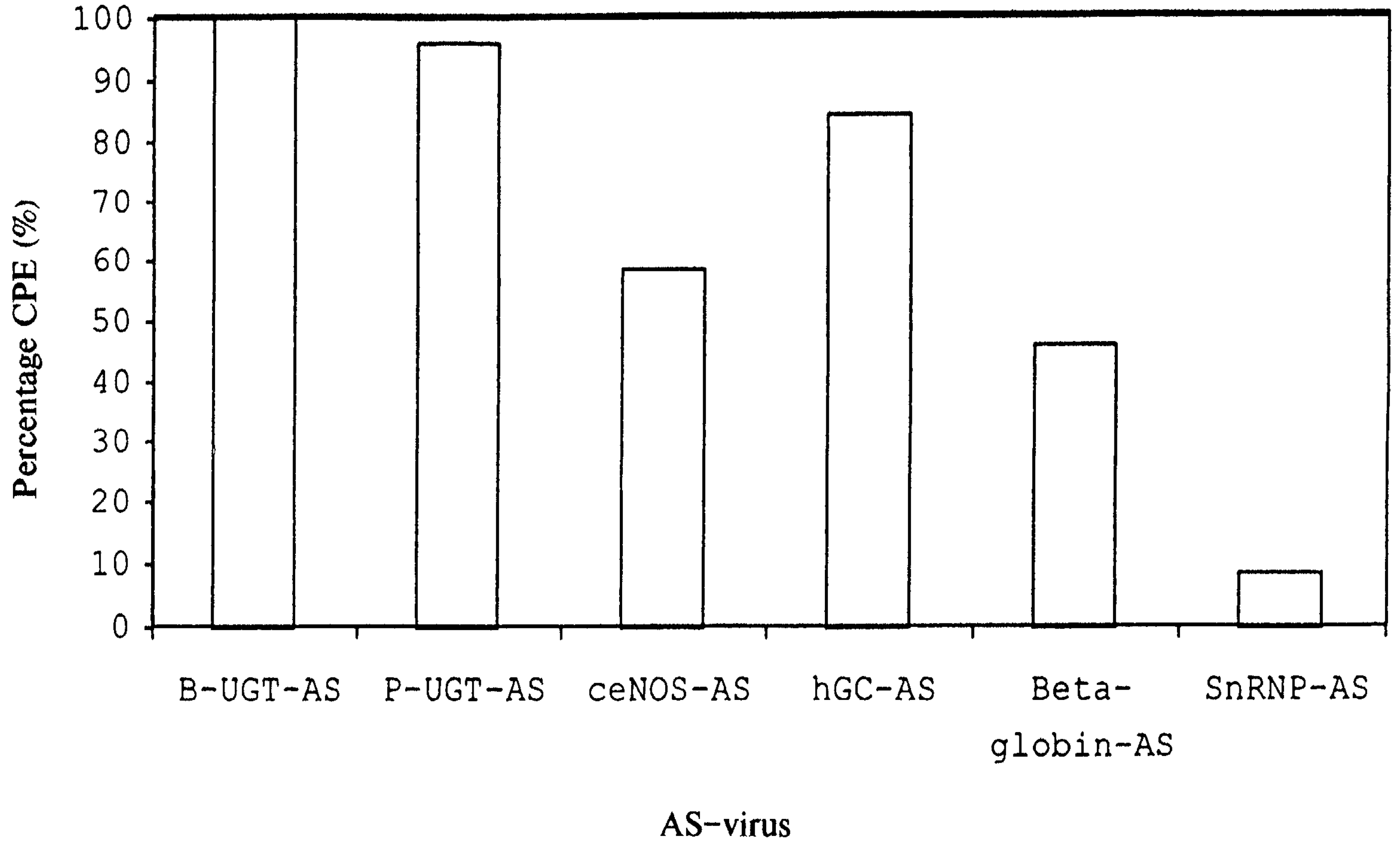

Percentage CPE (%)
100
90
80
70
60
50
40
30
20
10
0
B-UGT-AS
P-UGT-AS
ceNOS-AS
hGC-AS
Beta-
globin-AS
SnRNP-AS
AS-virus

FIG. 33

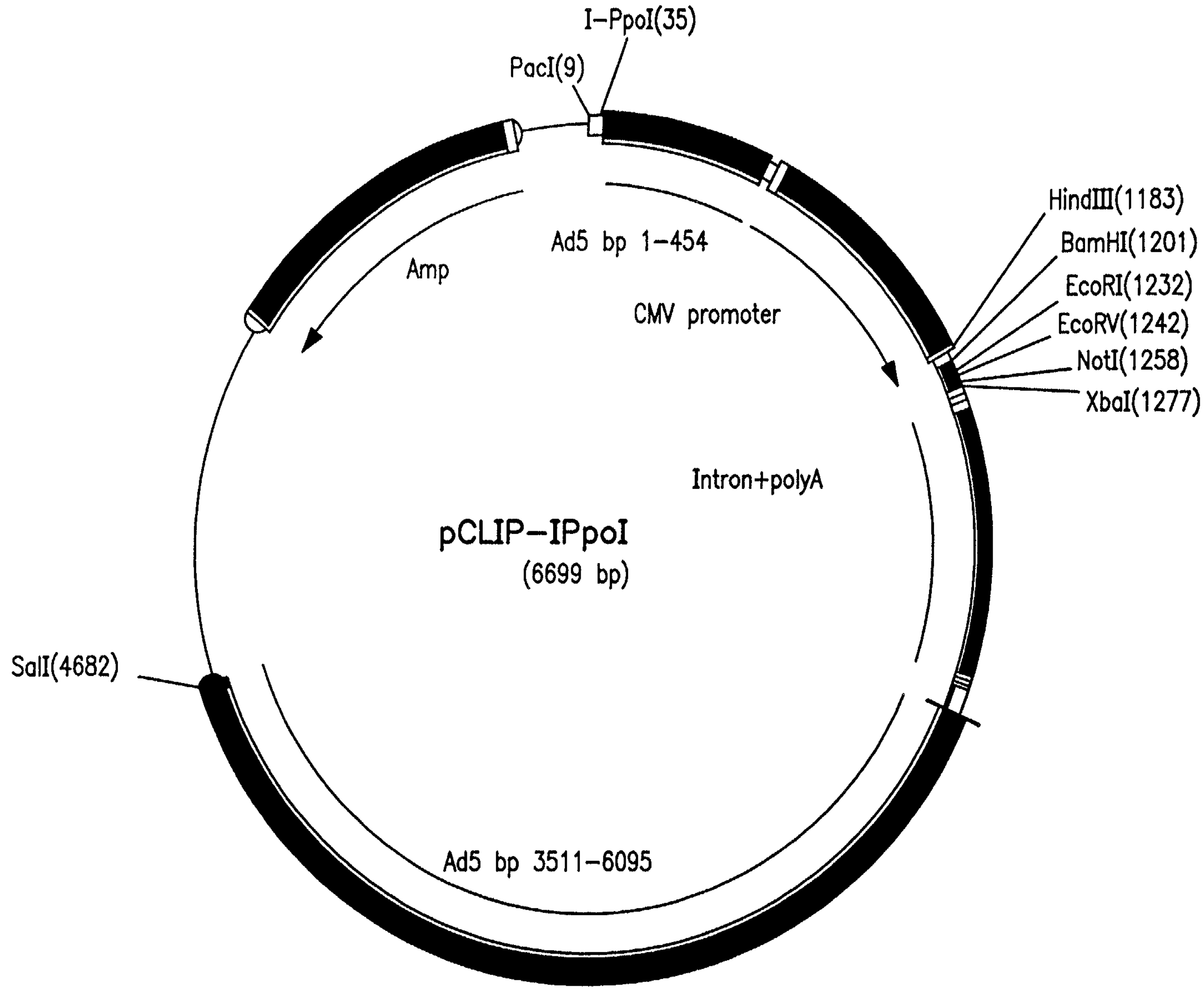

I-PpoI(35)
PacI(9)
HindIII(1183)
BamHI(1201)
EcoRI(1232)
EcoRV(1242)
NotI(1258)
XbaI(1277)
Ad5 bp 1-454
Amp
CMV promoter
Intron+polyA
pCLIP-IPpoI
(6699 bp)
SalI(4682)
Ad5 bp 3511-6095

FIG. 34A

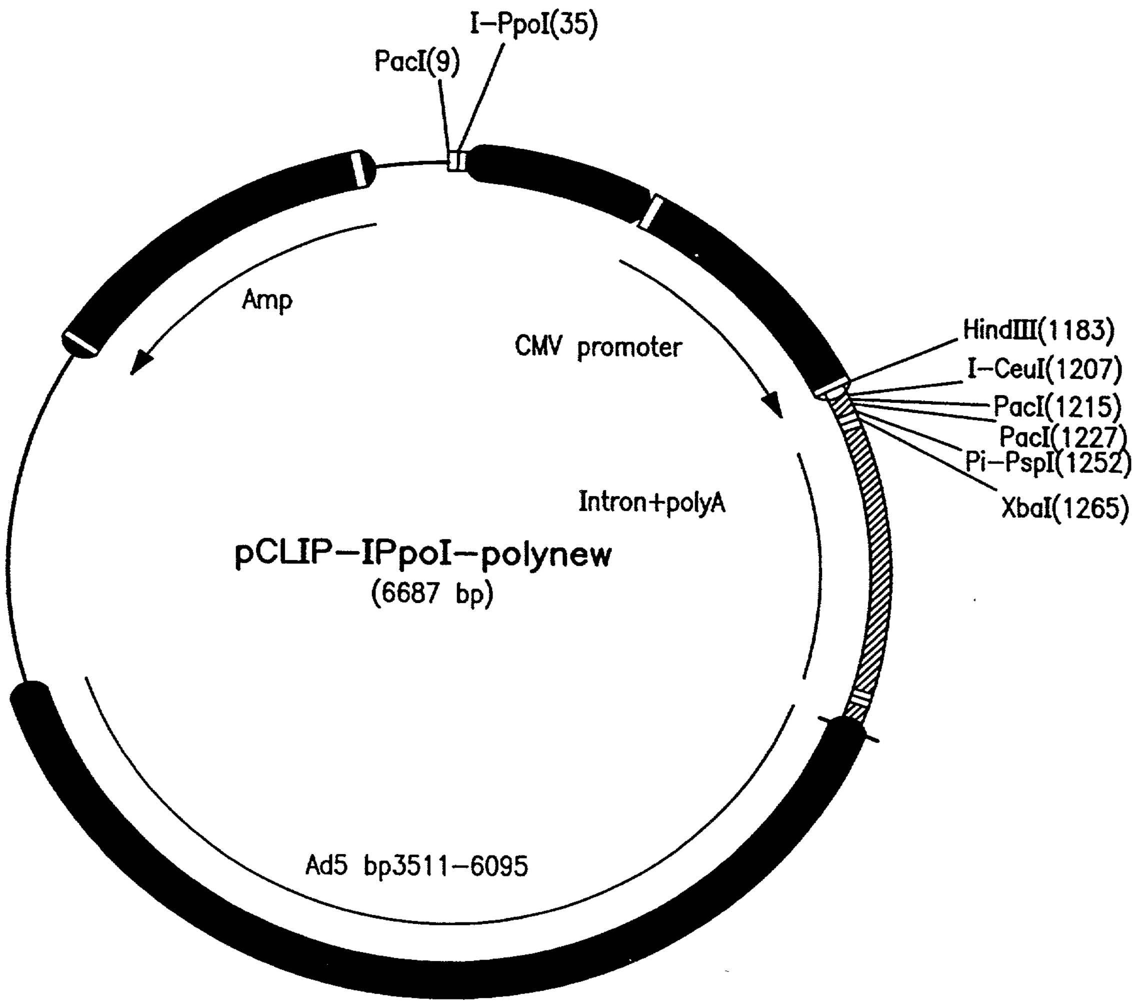

I-PpoI(35)
PacI(9)
Amp
CMV promoter
HindIII(1183)
I-CeuI(1207)
PacI(1215)
PacI(1227)
Pi-PspI(1252)
XbaI(1265)
Intron+polyA
pCLIP-IPpoI-polynew
(6687 bp)
Ad5 bp3511-6095

FIG. 34B

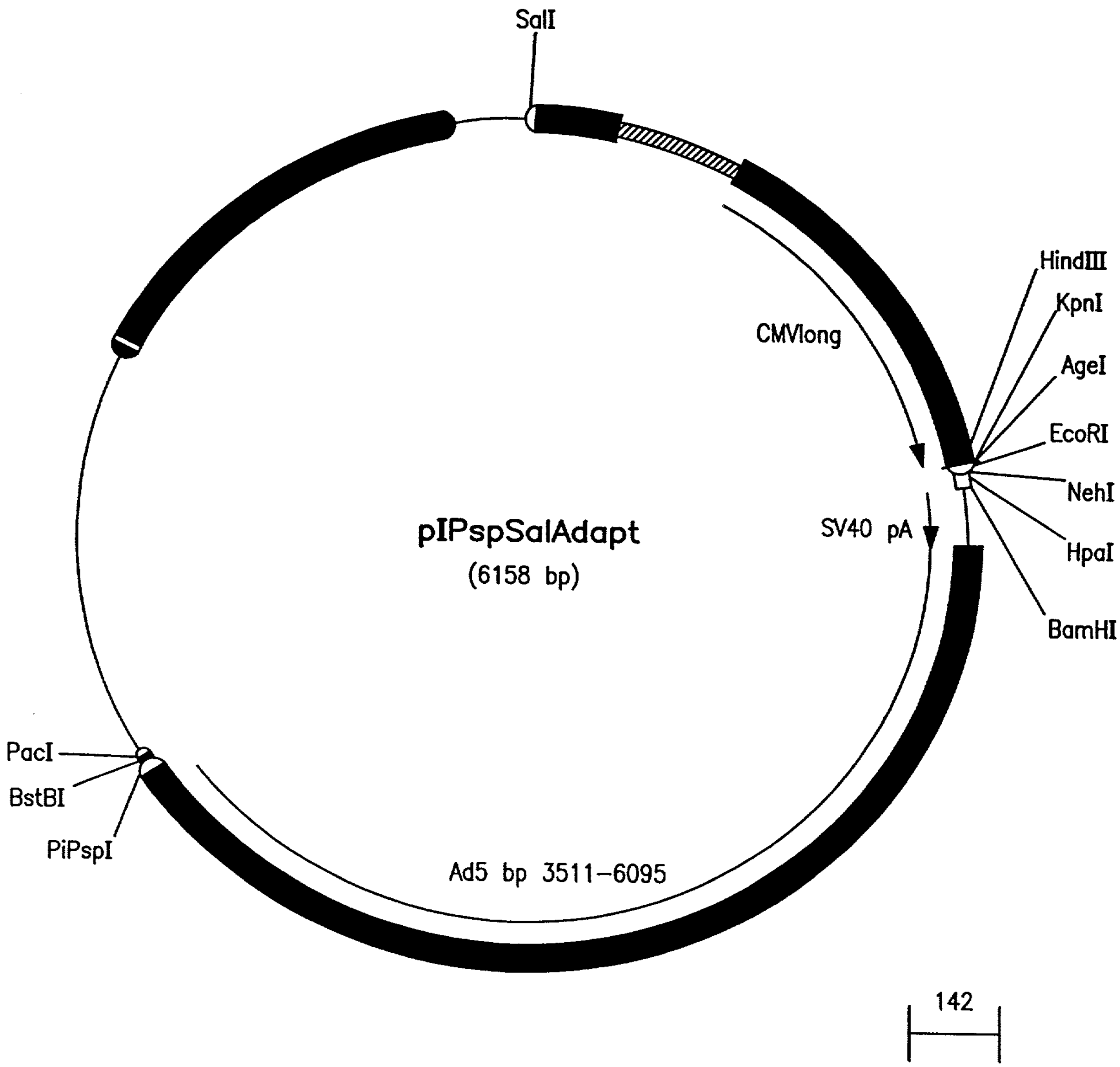

SalI
HindIII
KpnI
AgeI
EcoRI
NehI
HpaI
BamHI
CMVlong
SV40 pA
pIPspSalAdapt
(6158 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095
142

FIG. 34C

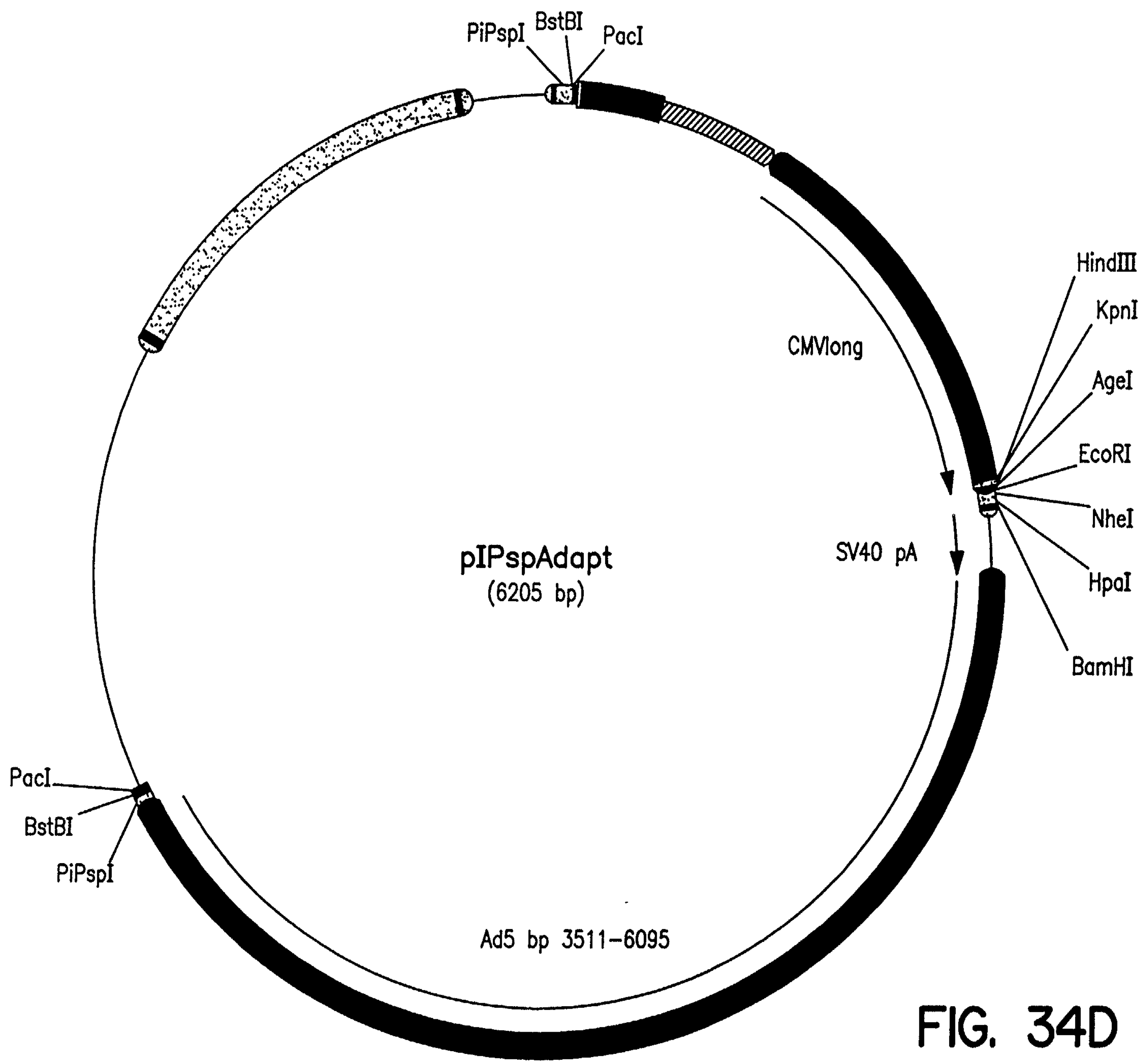

PiPspI
BstBI
PacI
HindIII
KpnI
AgeI
EcoRI
NheI
HpaI
BamHI
CMVlong
SV40 pA
pIPspAdapt
(6205 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095

FIG. 34D

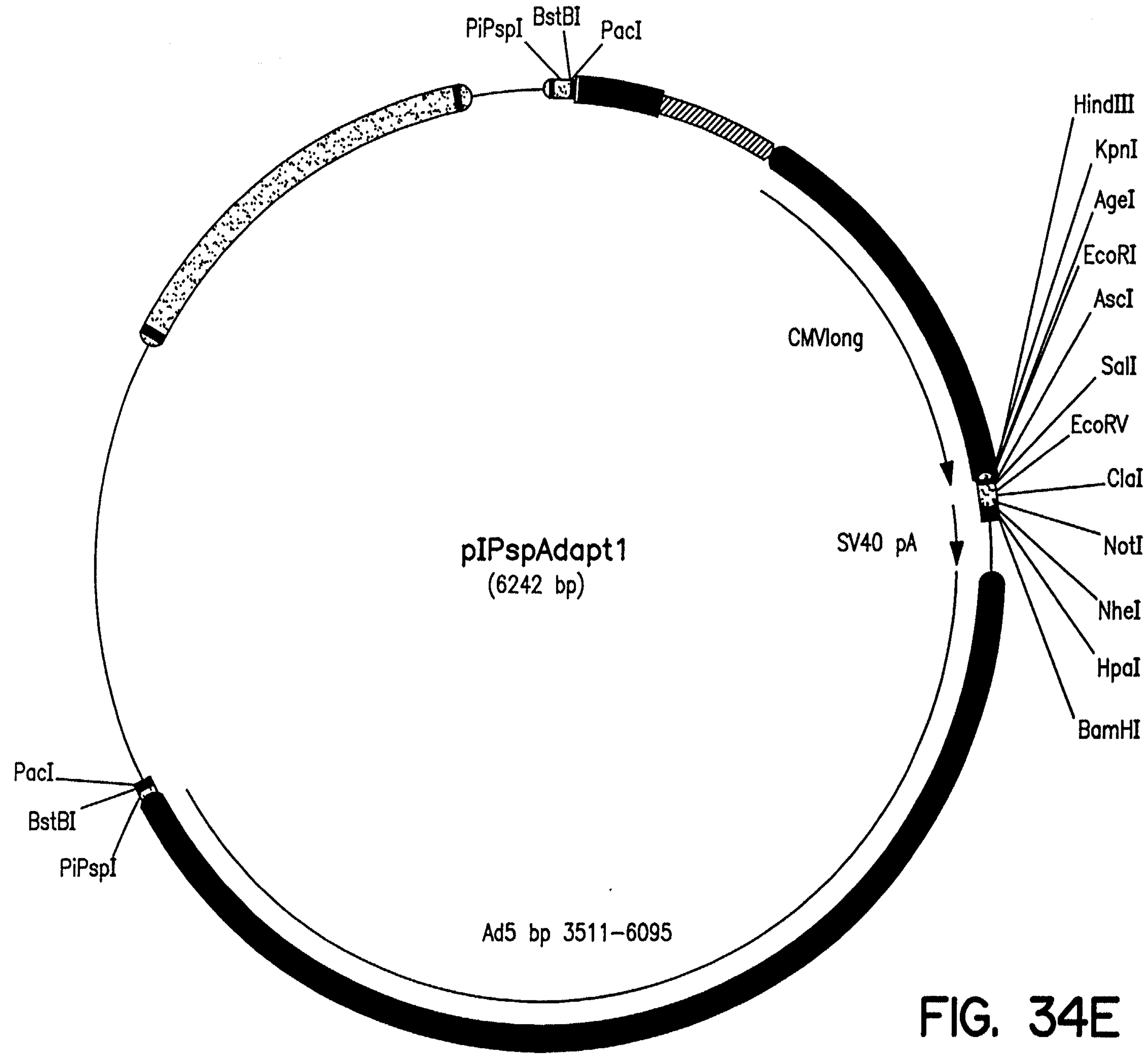

PiPspI
BstBI
PacI
HindIII
KpnI
AgeI
EcoRI
AscI
SalI
EcoRV
ClaI
NotI
NheI
HpaI
BamHI
CMVlong
SV40 pA
pIPspAdapt1
(6242 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095

FIG. 34E

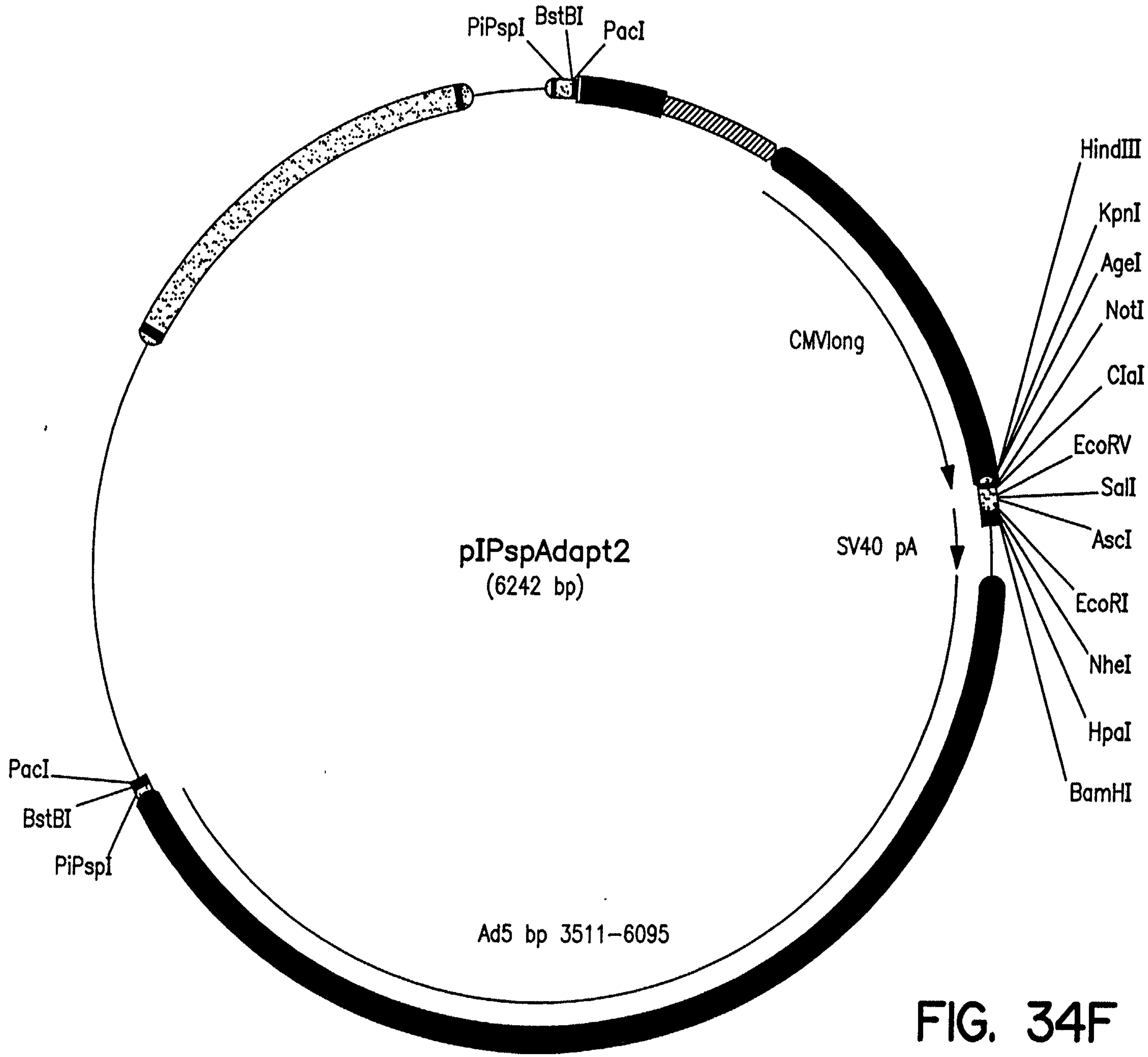

PiPspI
BstBI
PacI
HindIII
KpnI
AgeI
NotI
ClaI
EcoRV
SalI
AscI
EcoRI
NheI
HpaI
BamHI
CMVlong
SV40 pA
pIPspAdapt2
(6242 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095

FIG. 34F

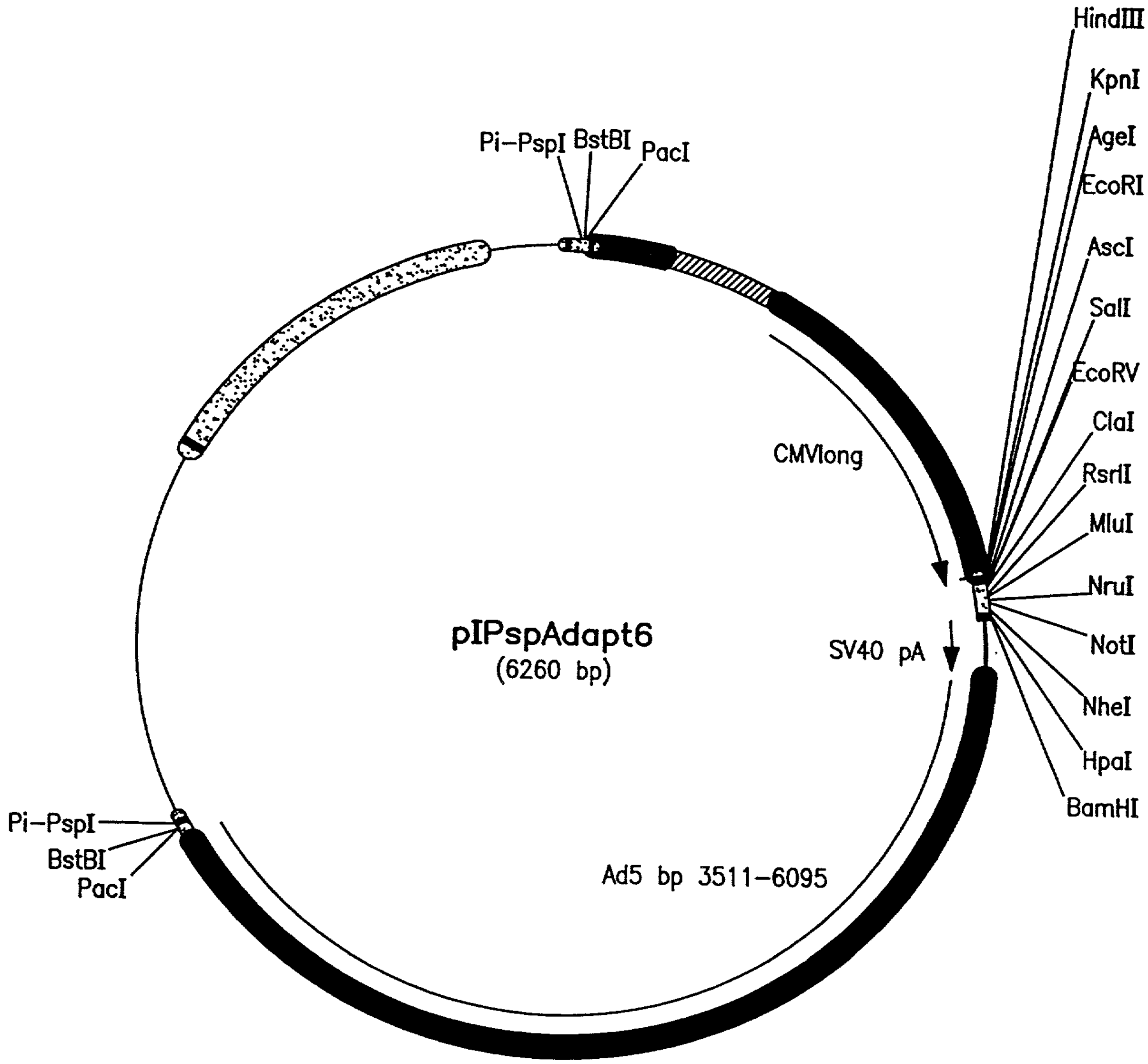

pIPspAdapt6
(6260 bp)
Pi-PspI
BstBI
PacI
CMVlong
SV40 pA
Ad5 bp 3511-6095
HindIII
KpnI
AgeI
EcoRI
AscI
SalI
EcoRV
ClaI
RsrII
MluI
NruI
NotI
NheI
HpaI
BamHI
Pi-PspI
BstBI
PacI

FIG. 34G

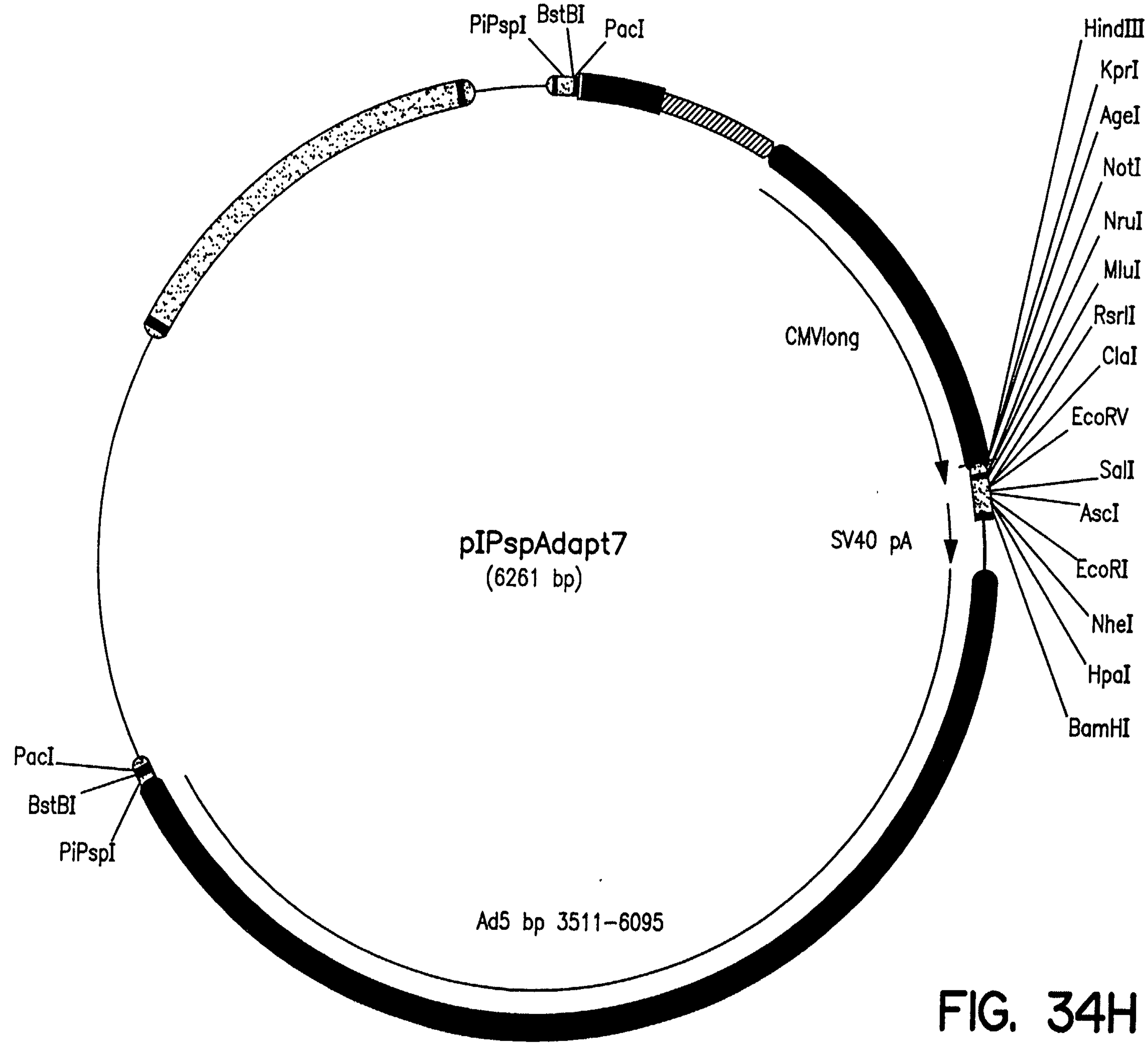

PiPspI
BstBI
PacI
HindIII
KprI
AgeI
NotI
NruI
MluI
RsrII
ClaI
EcoRV
SalI
AscI
EcoRI
NheI
HpaI
BamHI
CMVlong
SV40 pA
pIPspAdapt7
(6261 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095

FIG. 34H

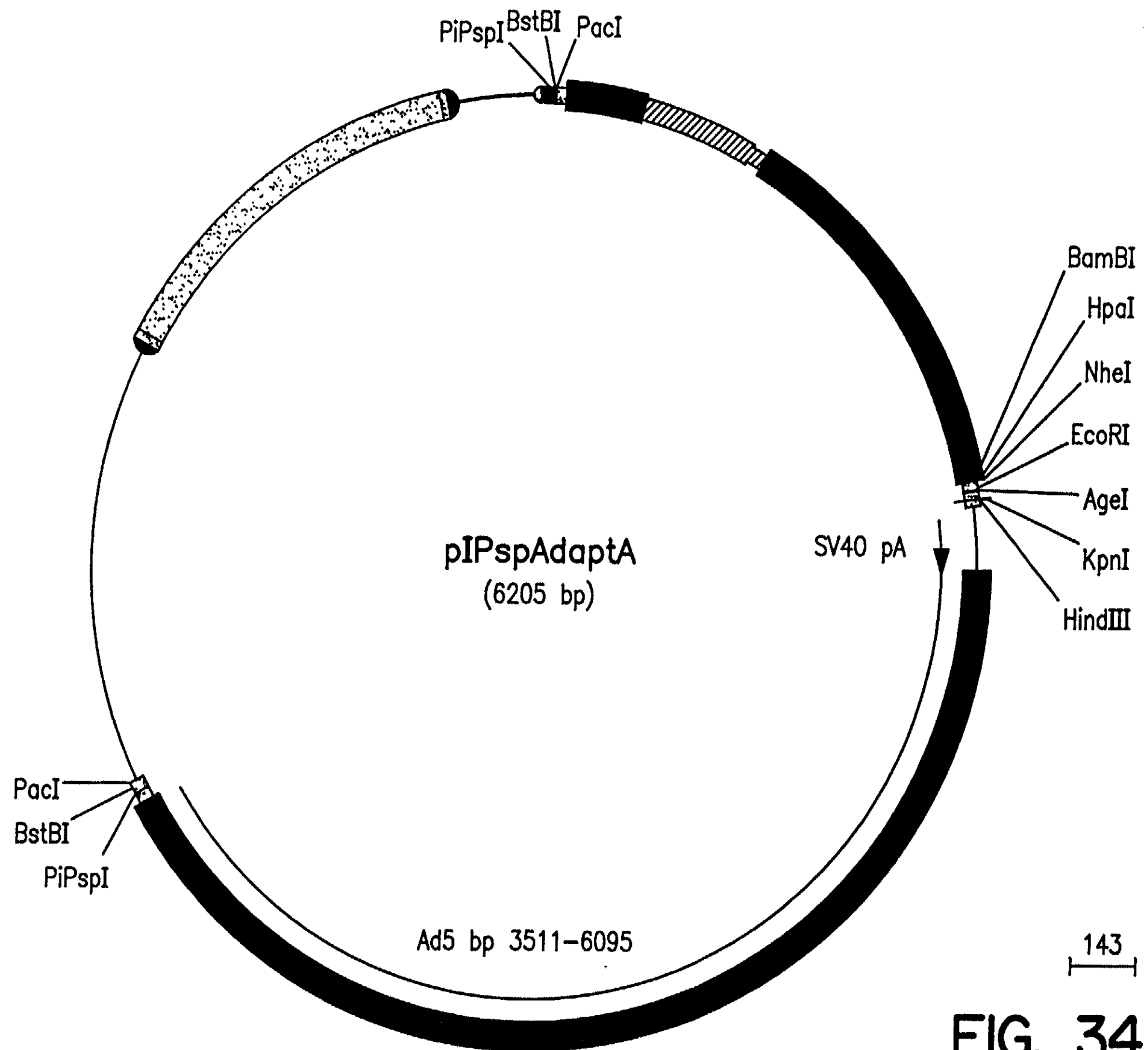

PiPspI
BstBI
PacI
BamBI
HpaI
NheI
EcoRI
AgeI
KpnI
HindIII
pIPspAdaptA
(6205 bp)
SV40 pA
PacI
BstBI
PiPspI
Ad5 bp 3511-6095
143

FIG. 34 I

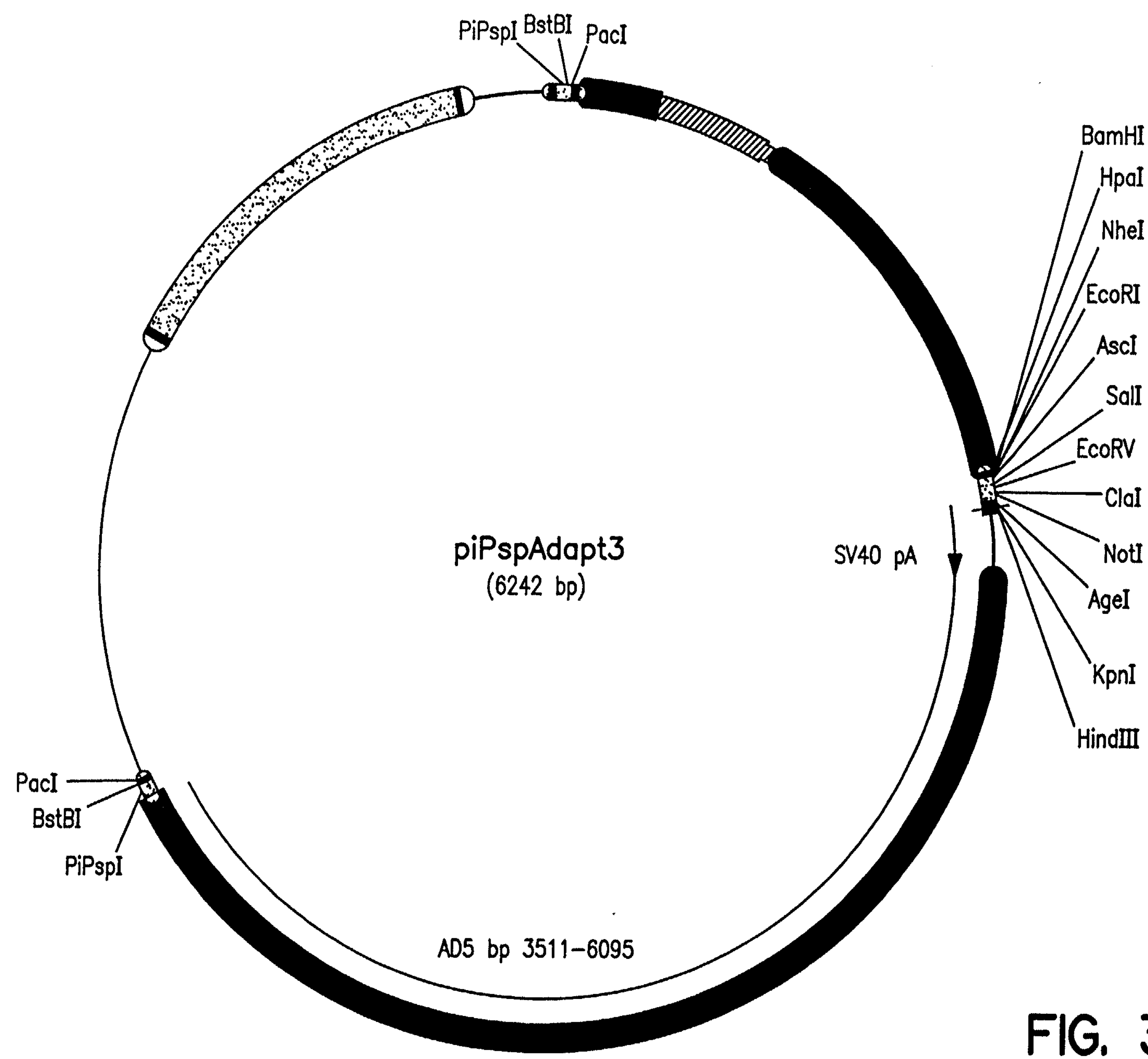

piPspAdapt3
(6242 bp)
PiPspI
BstBI
PacI
BamHI
HpaI
NheI
EcoRI
AscI
SalI
EcoRV
ClaI
NotI
AgeI
KpnI
HindIII
SV40 pA
PacI
BstBI
PiPspI
AD5 bp 3511-6095

FIG. 34J

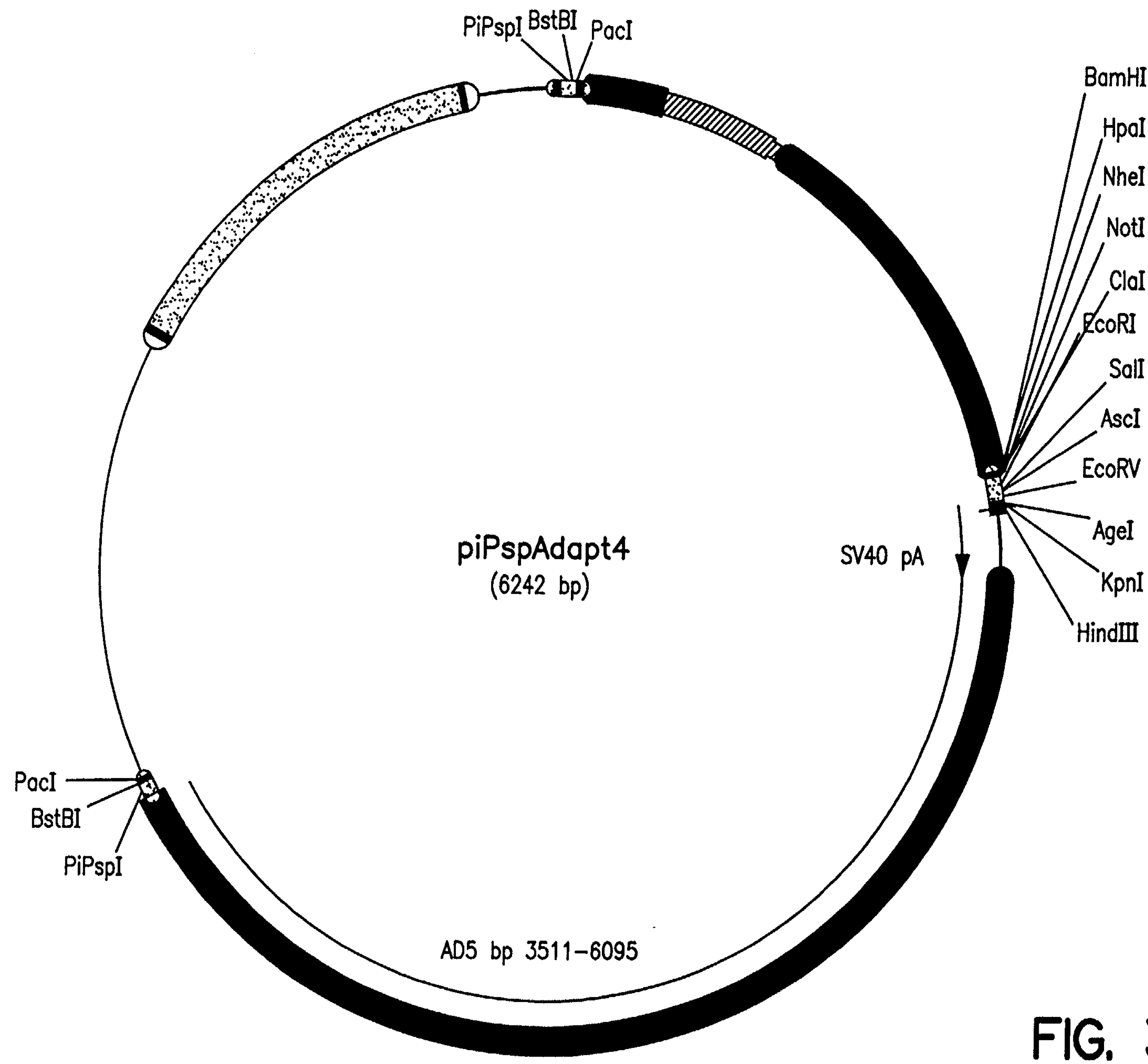
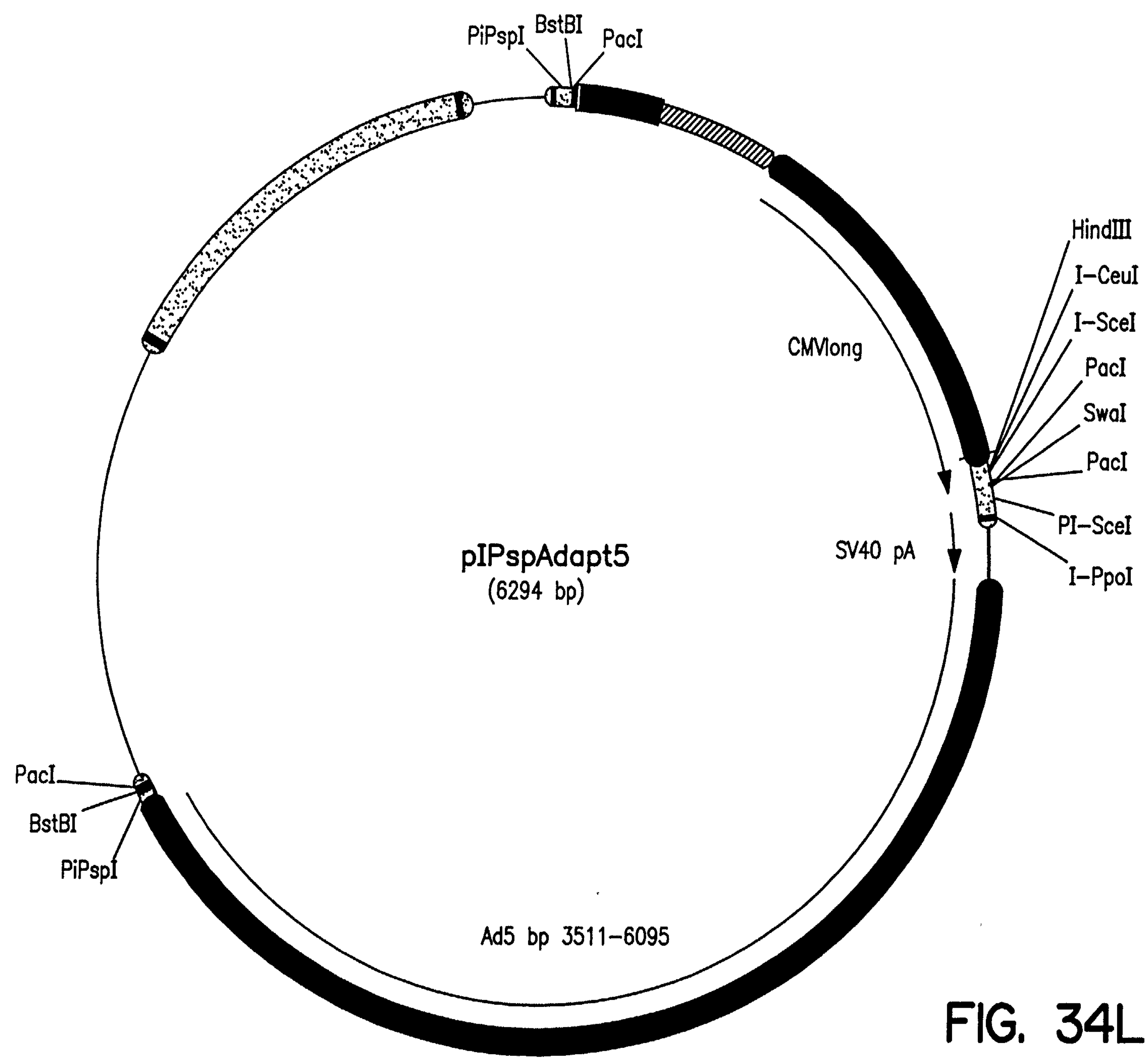

PiPspI
BstBI
PacI
BamHI
HpaI
NheI
NotI
ClaI
EcoRI
SalI
AscI
EcoRV
AgeI
KpnI
HindIII
SV40 pA
piPspAdapt4
(6242 bp)
PacI
BstBI
PiPspI
AD5 bp 3511-6095

FIG. 34K



PiPspI
BstBI
PacI
HindIII
I-CeuI
I-SceI
PacI
SwaI
PacI
PI-SceI
I-PpoI
CMVlong
SV40 pA
pIPspAdapt5
(6294 bp)
PacI
BstBI
PiPspI
Ad5 bp 3511-6095

FIG. 34L

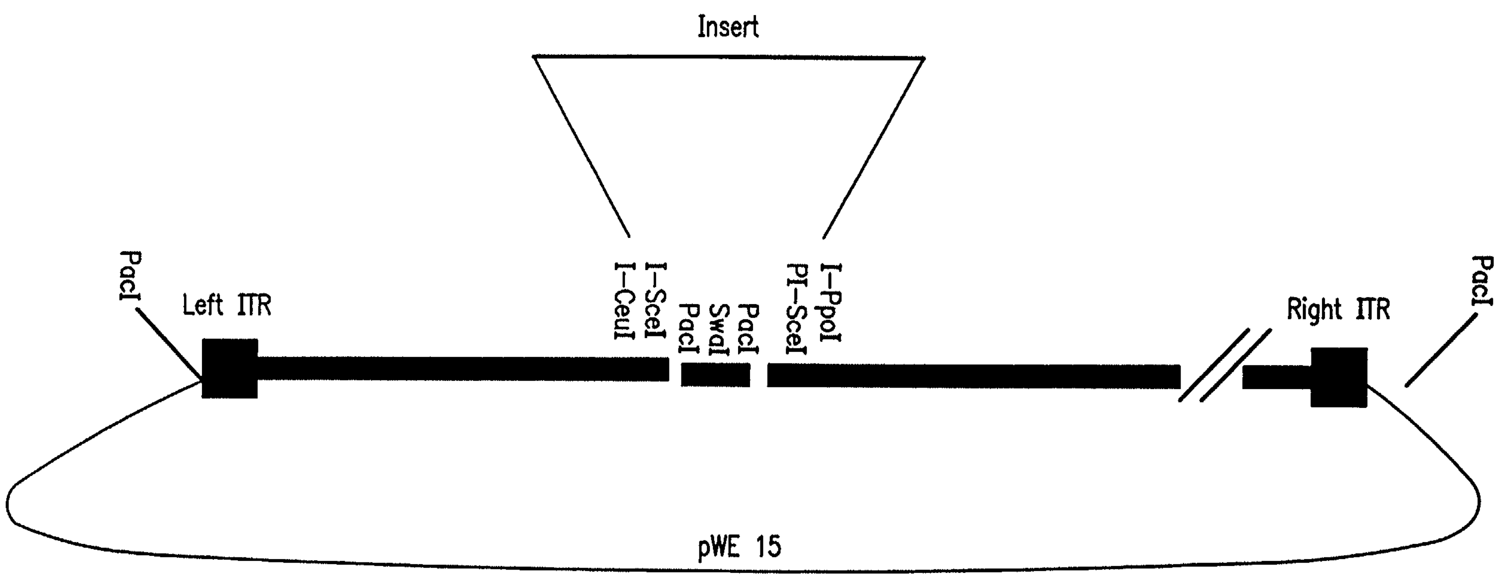

Insert
PacI
Left ITR
I-SceI
I-CeuI
PacI
SwaI
PacI
I-PpoI
PI-SceI
Right ITR
PacI
pWE 15

FIG. 34M

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

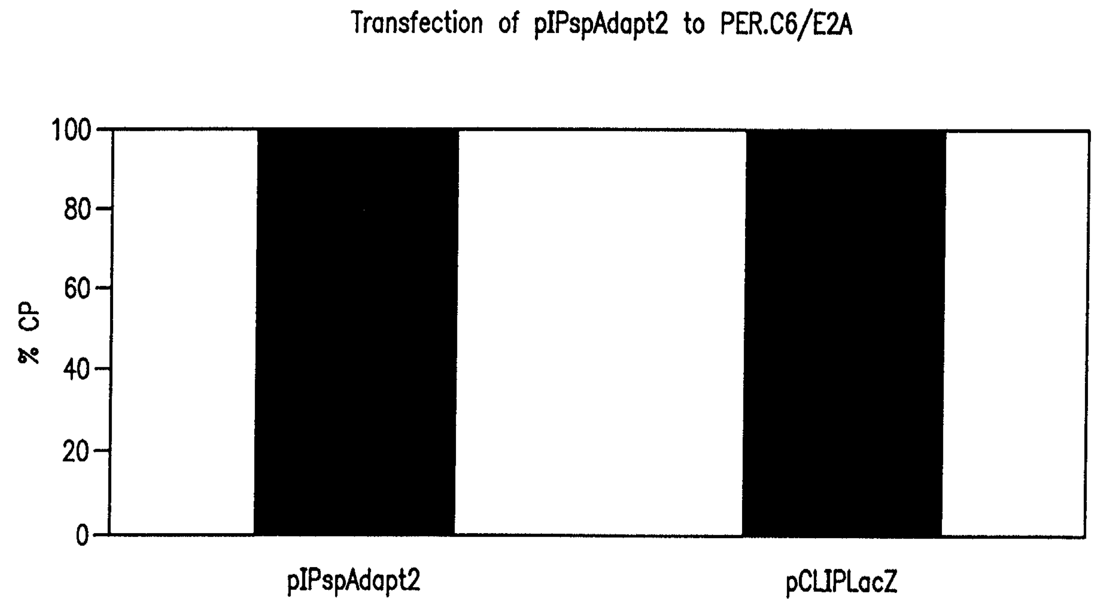


FIG. 34N

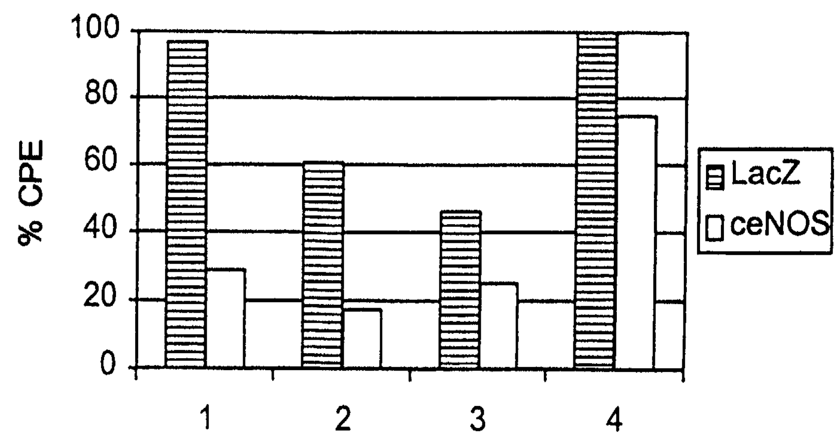


FIG. 35

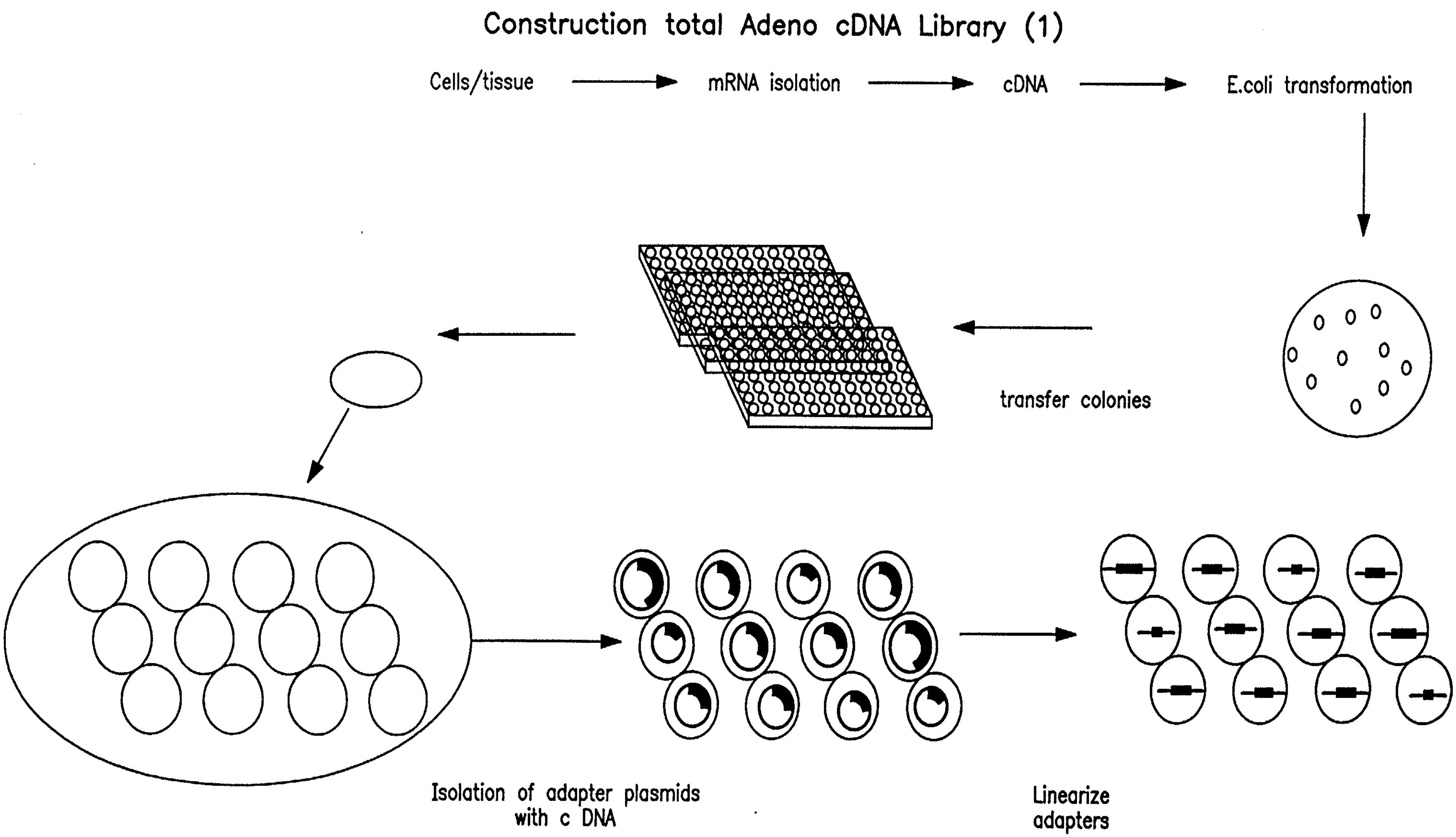

Construction total Adeno cDNA Library (1)
Cells/tissue
mRNA isolation
cDNA
E.coli transformation
transfer colonies
Isolation of adapter plasmids with c DNA
Linearize adapters

FIG. 36A

Construction total Adeno cDNA Library (II)

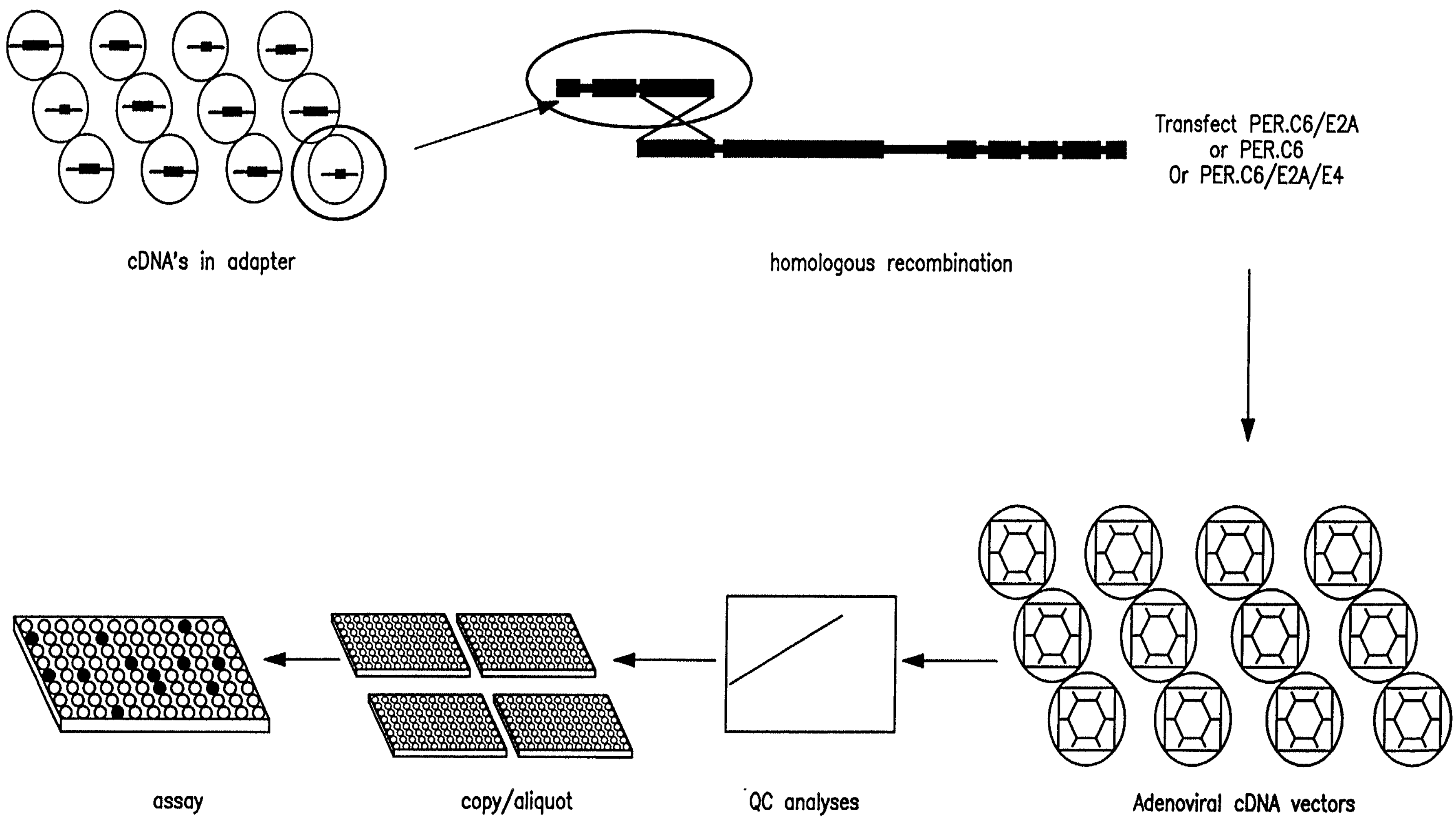


FIG. 36B

EXAMPLE 21 384 WELL PLATE IN PROGRESS

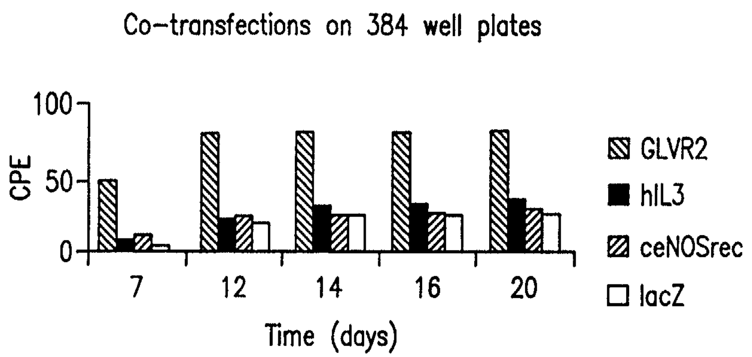


FIG. 37A

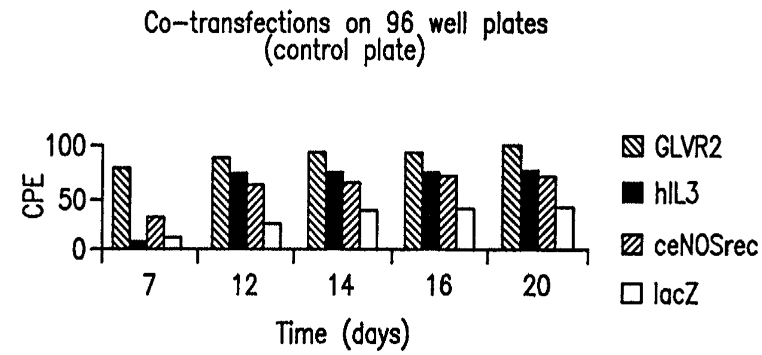


FIG. 37B

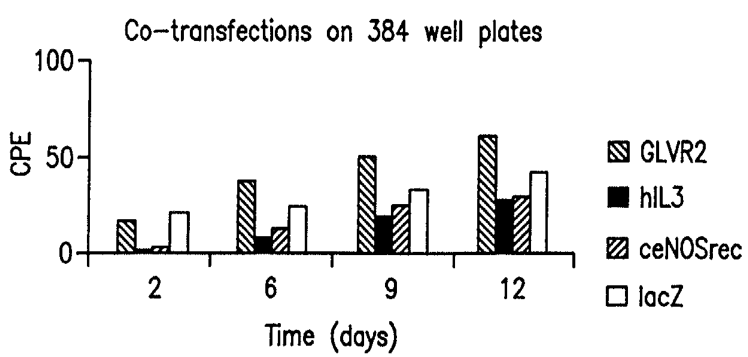


FIG. 37C

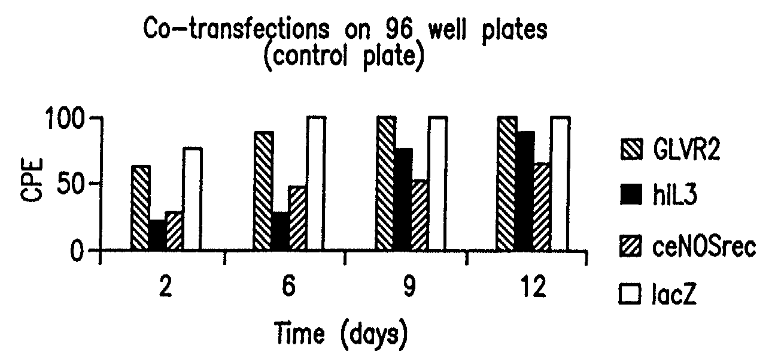


FIG. 37D

Medium changed 7 days after transfection

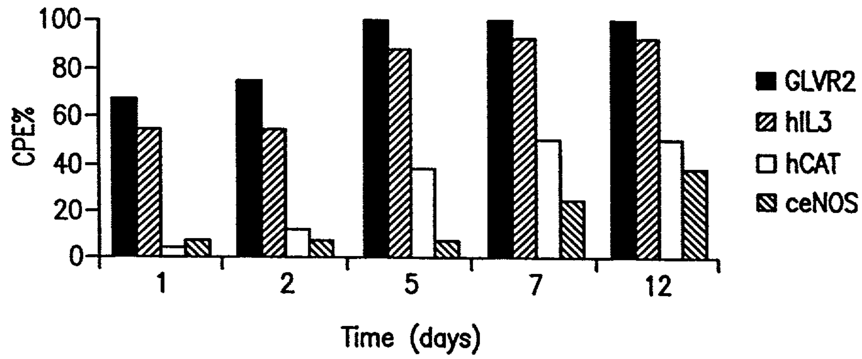


FIG. 38A

Medium not changed

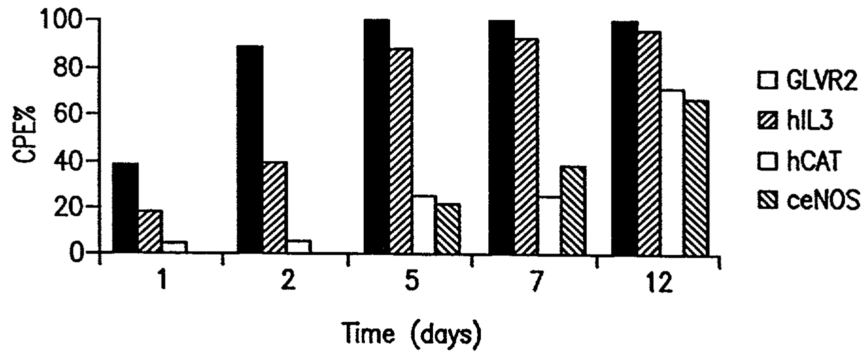


FIG. 38B

Propagation 7 days after transfection

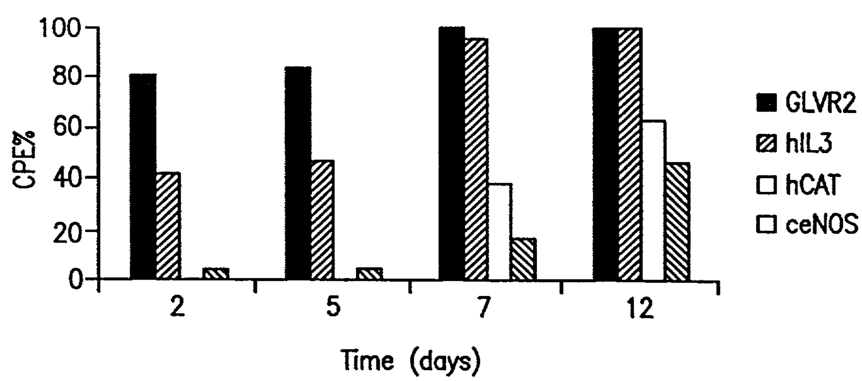


FIG. 38C

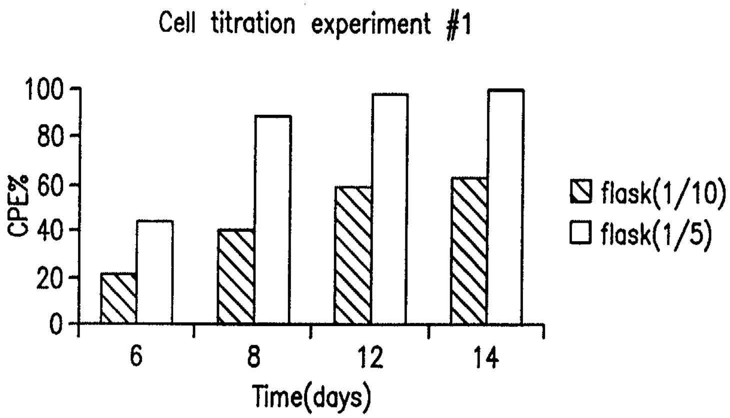


FIG. 39A

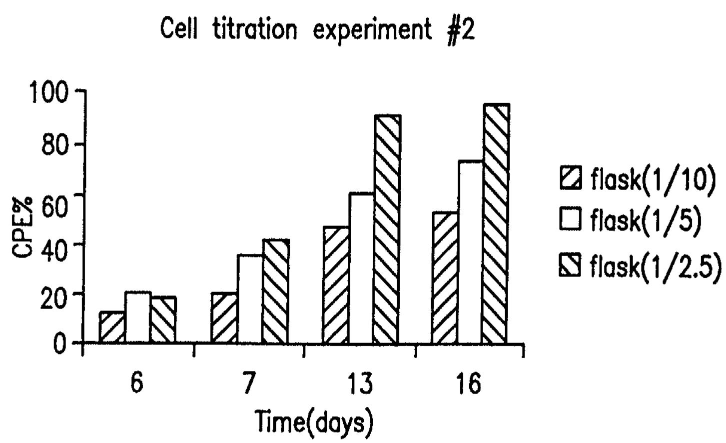


FIG. 39B

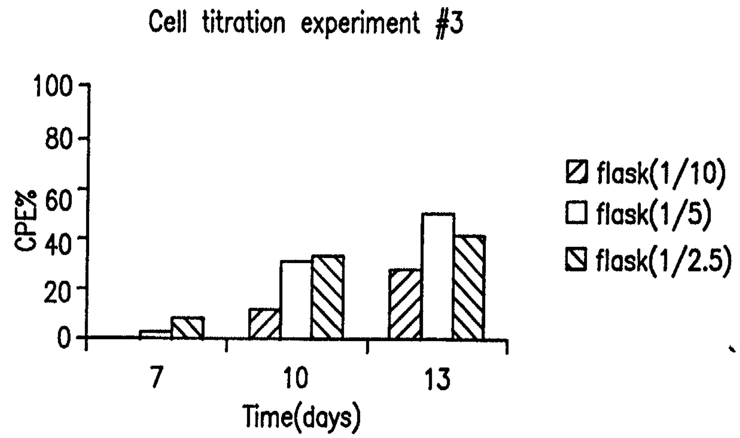


FIG. 39C

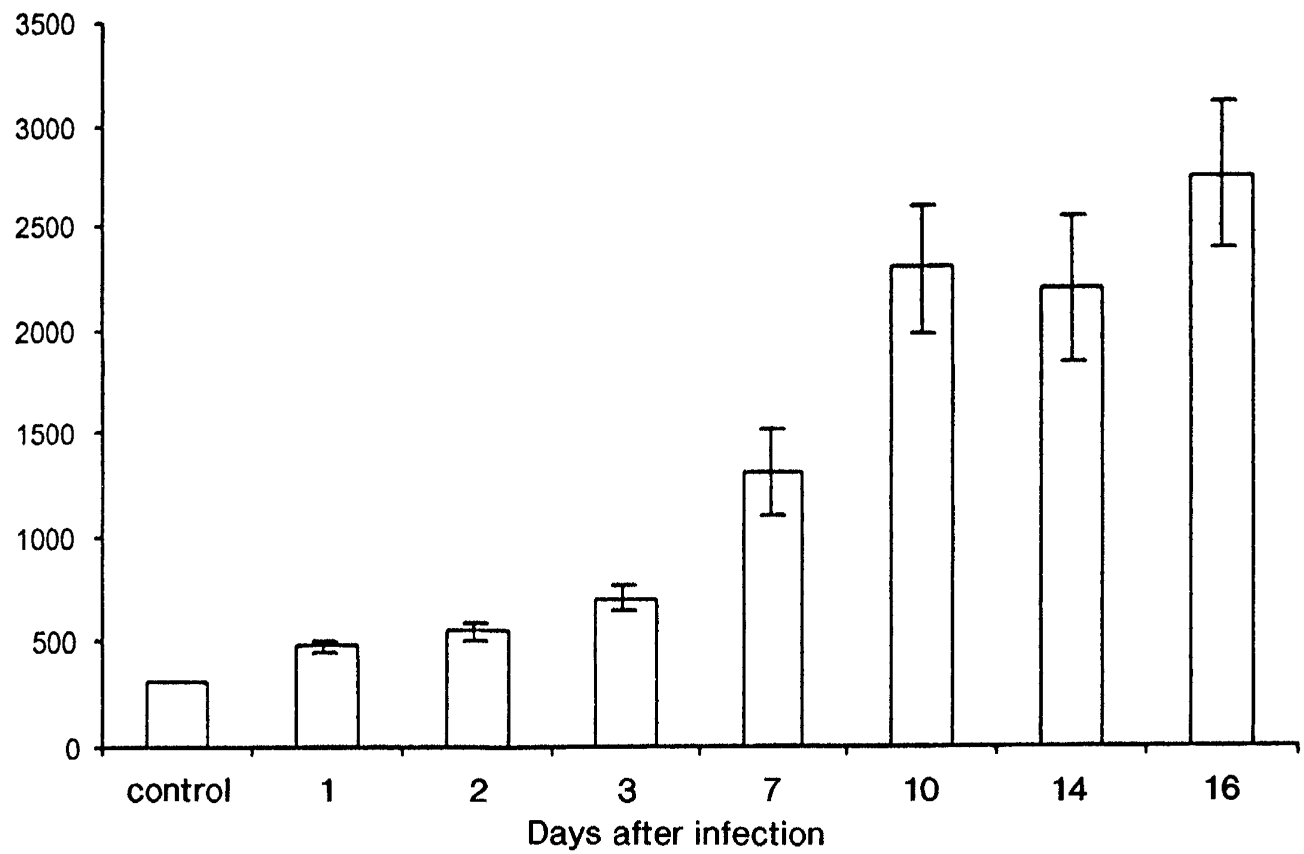

3500
3000
2500
2000
1500
1000
500
0
control
1
2
3
7
10
14
16
Days after infection

FIG. 40

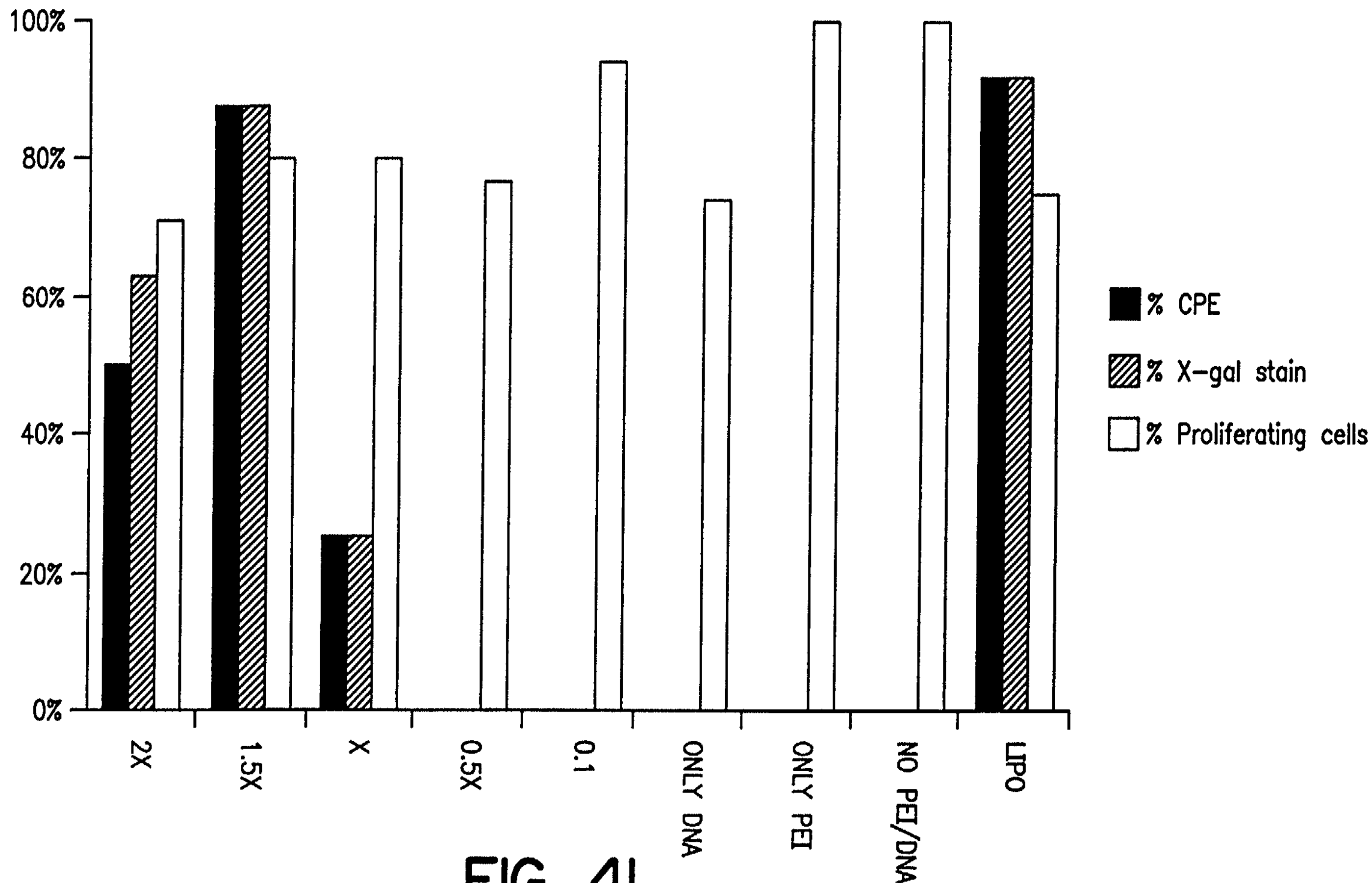

100%
80%
60%
40%
20%
0%
2X
1.5X
X
0.5X
0.1
ONLY DNA
ONLY PEI
NO PEI/DNA
LIPO
% CPE
% X-gal stain
% Proliferating cells

FIG. 4I

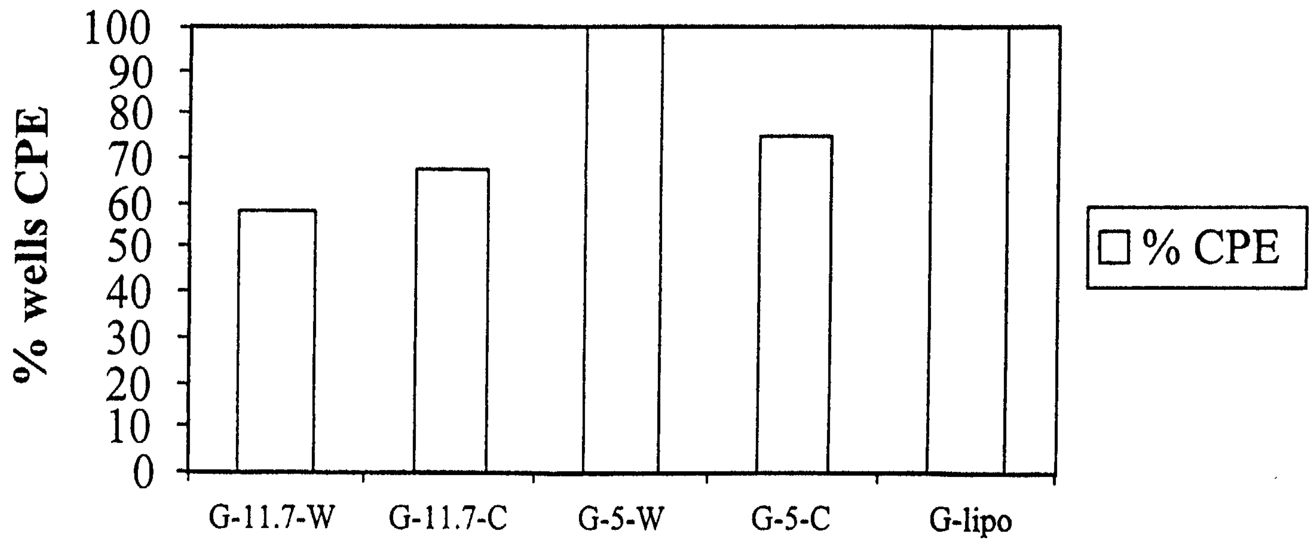

% wells CPE
100
90
80
70
60
50
40
30
20
10
0
G-11.7-W
G-11.7-C
G-5-W
G-5-C
G-lipo
% CPE

FIG. 42

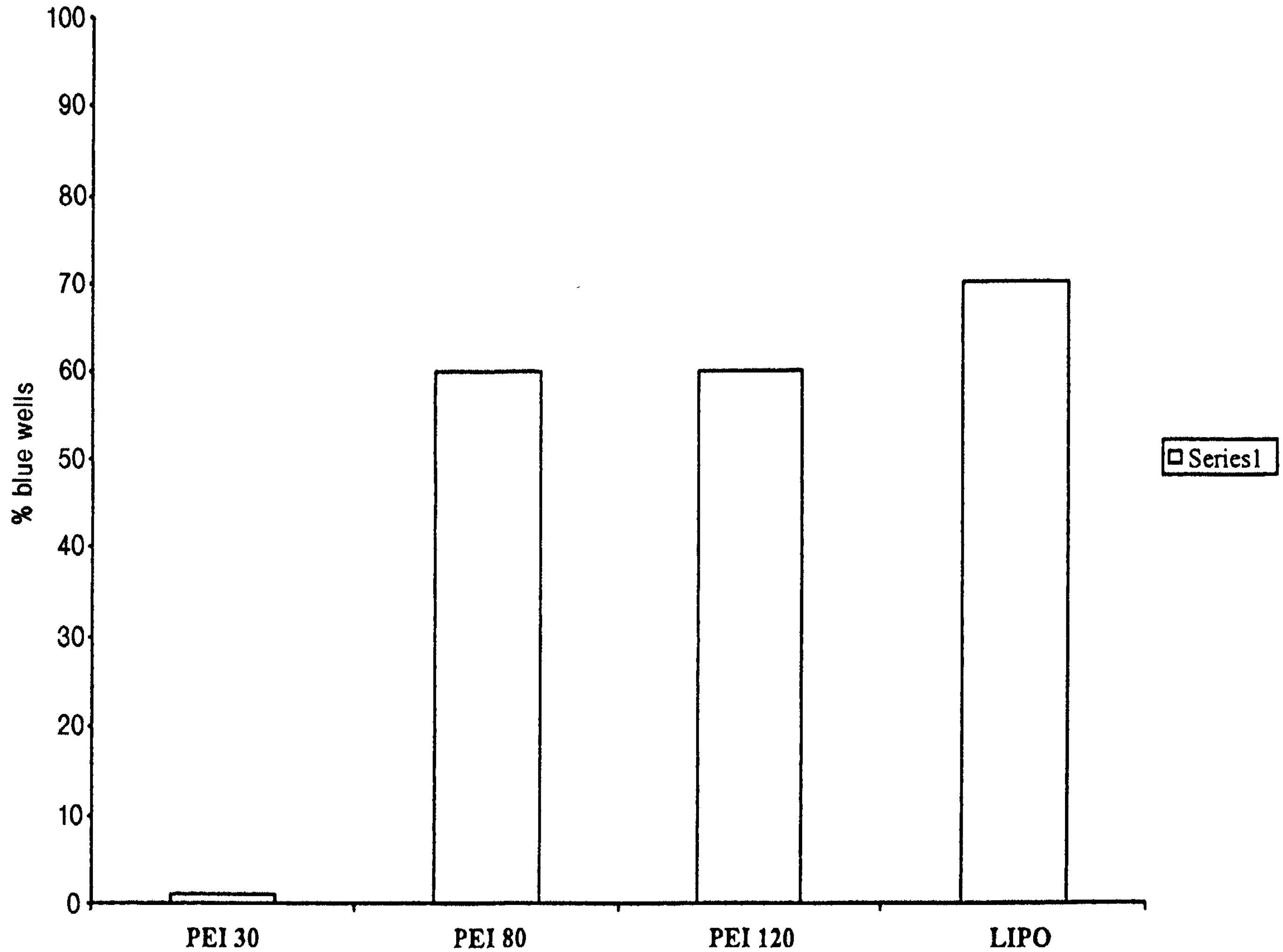

100
90
80
70
60
50
40
30
20
10
0
% blue wells
Series1
PEI 30
PEI 80
PEI 120
LIPO

FIG. 43

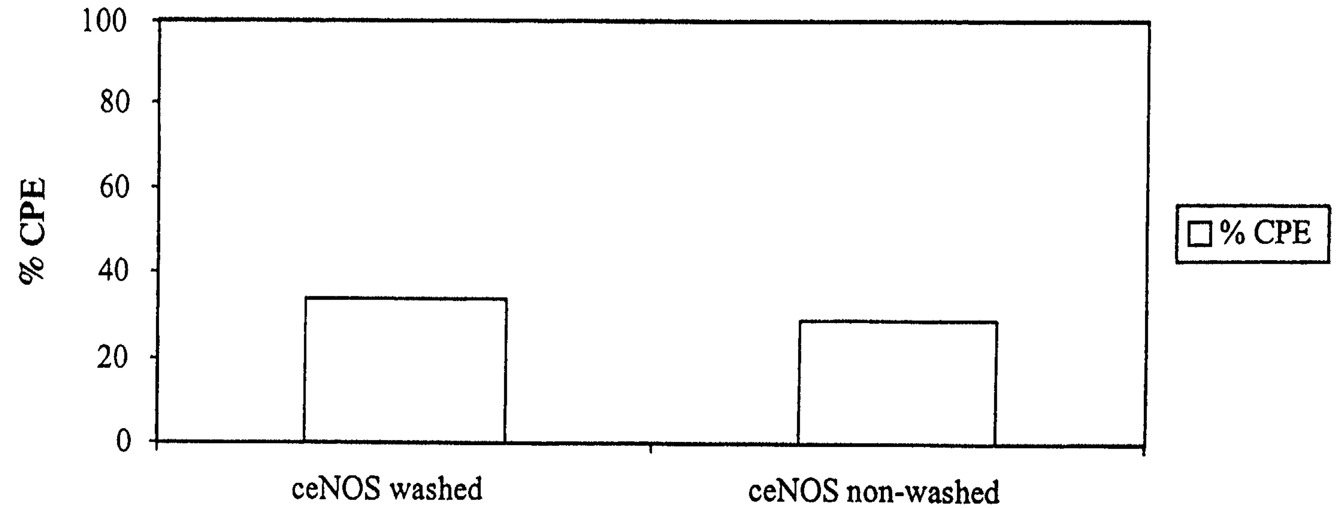


FIG. 44

Figure 45

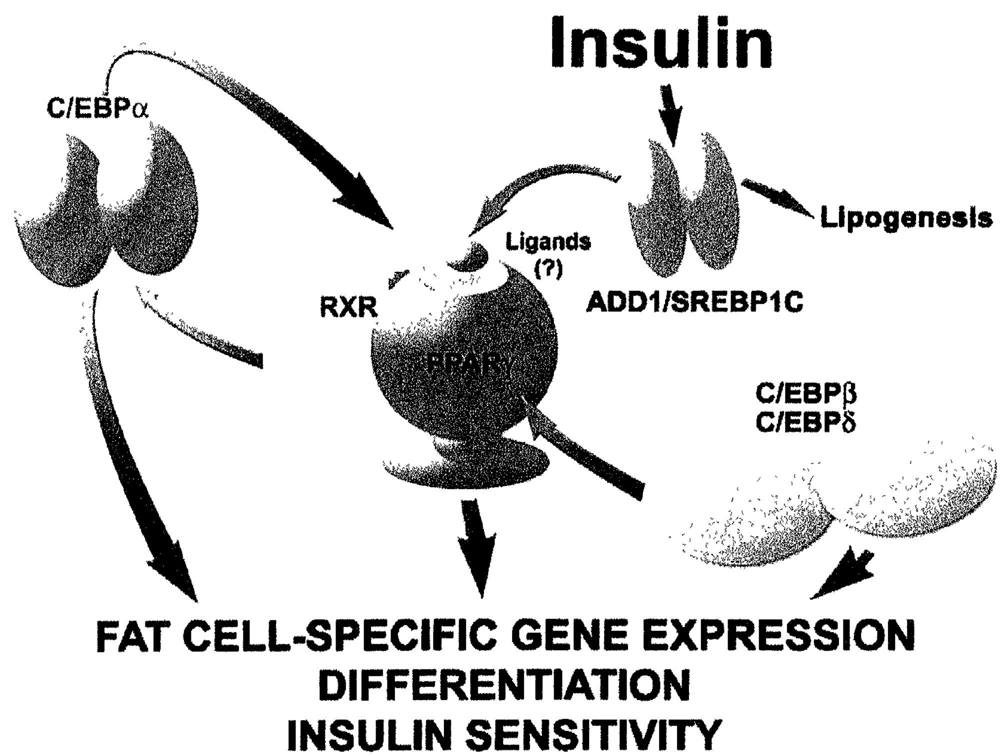

Insulin
C/EBPα
Ligands
(?)
RXR
PPARγ
ADD1/SREBP1C
Lipogenesis
C/EBPβ
C/EBPδ
FAT CELL-SPECIFIC GENE EXPRESSION
DIFFERENTIATION
INSULIN SENSITIVITY

Figure 46

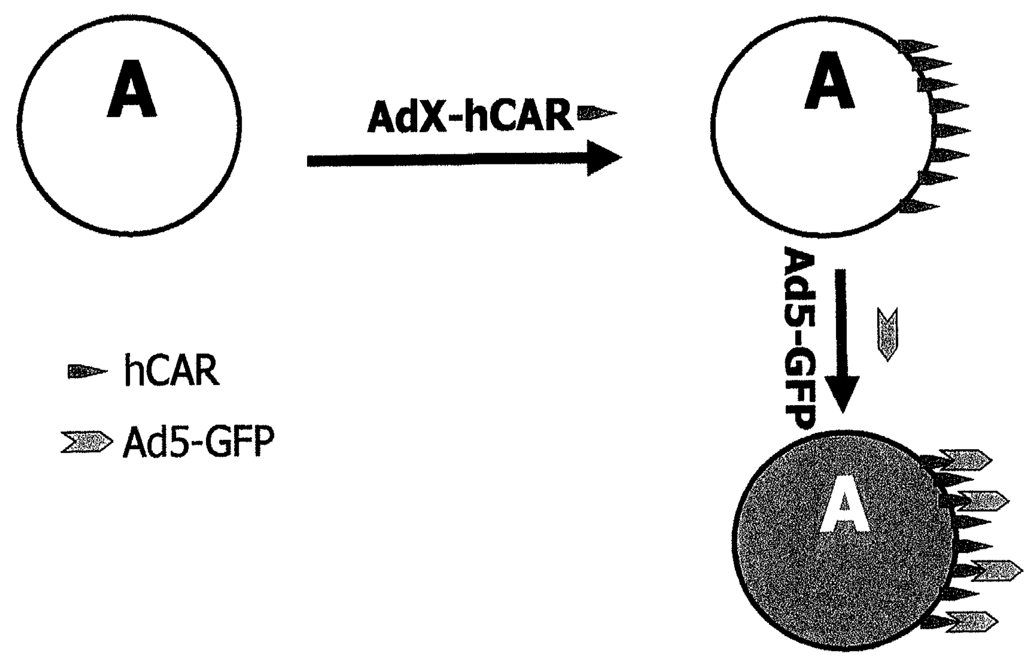


Figure 47

Infection of human primary pre-adipocytes using Ad5C01 and Ad5C20 fiber-modified viruses

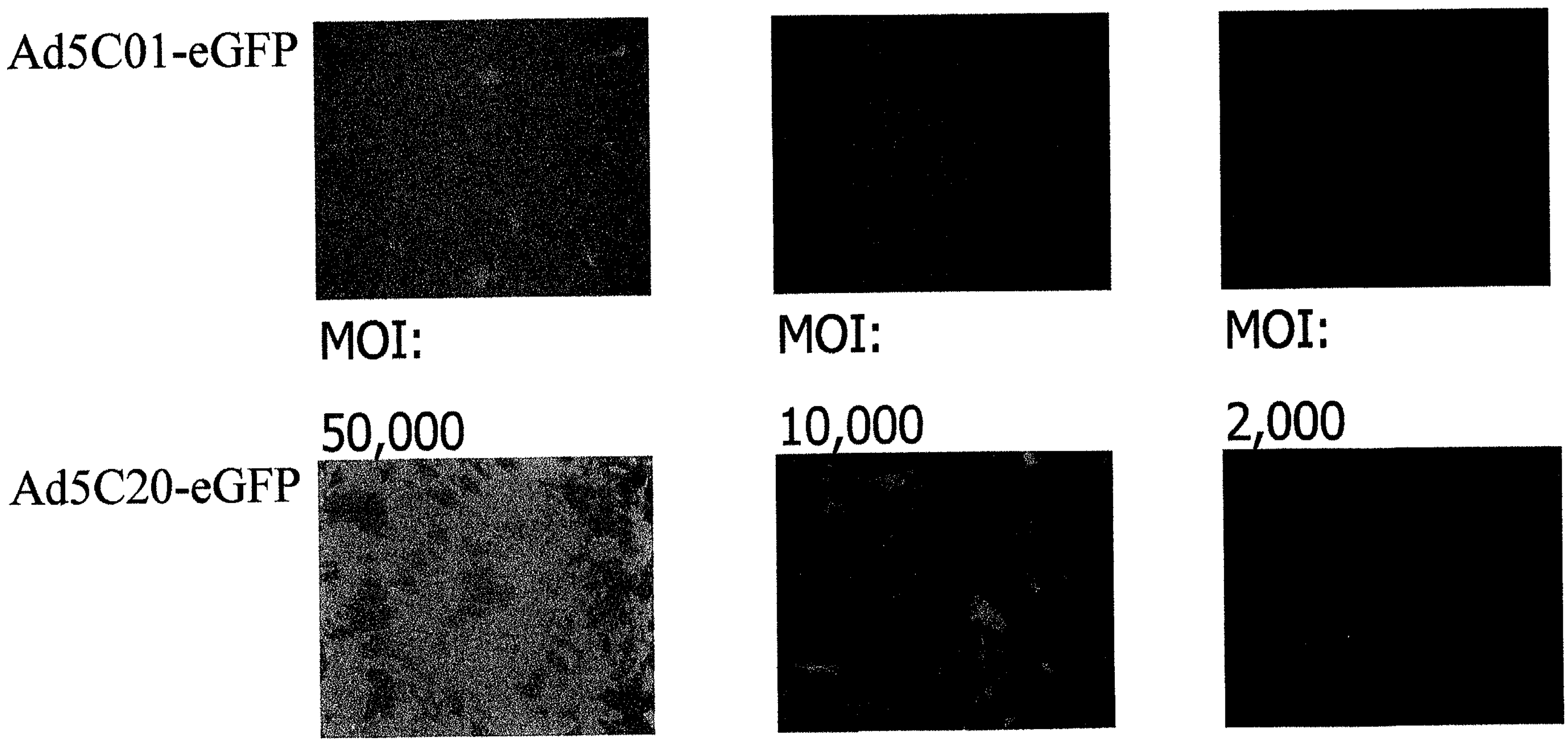
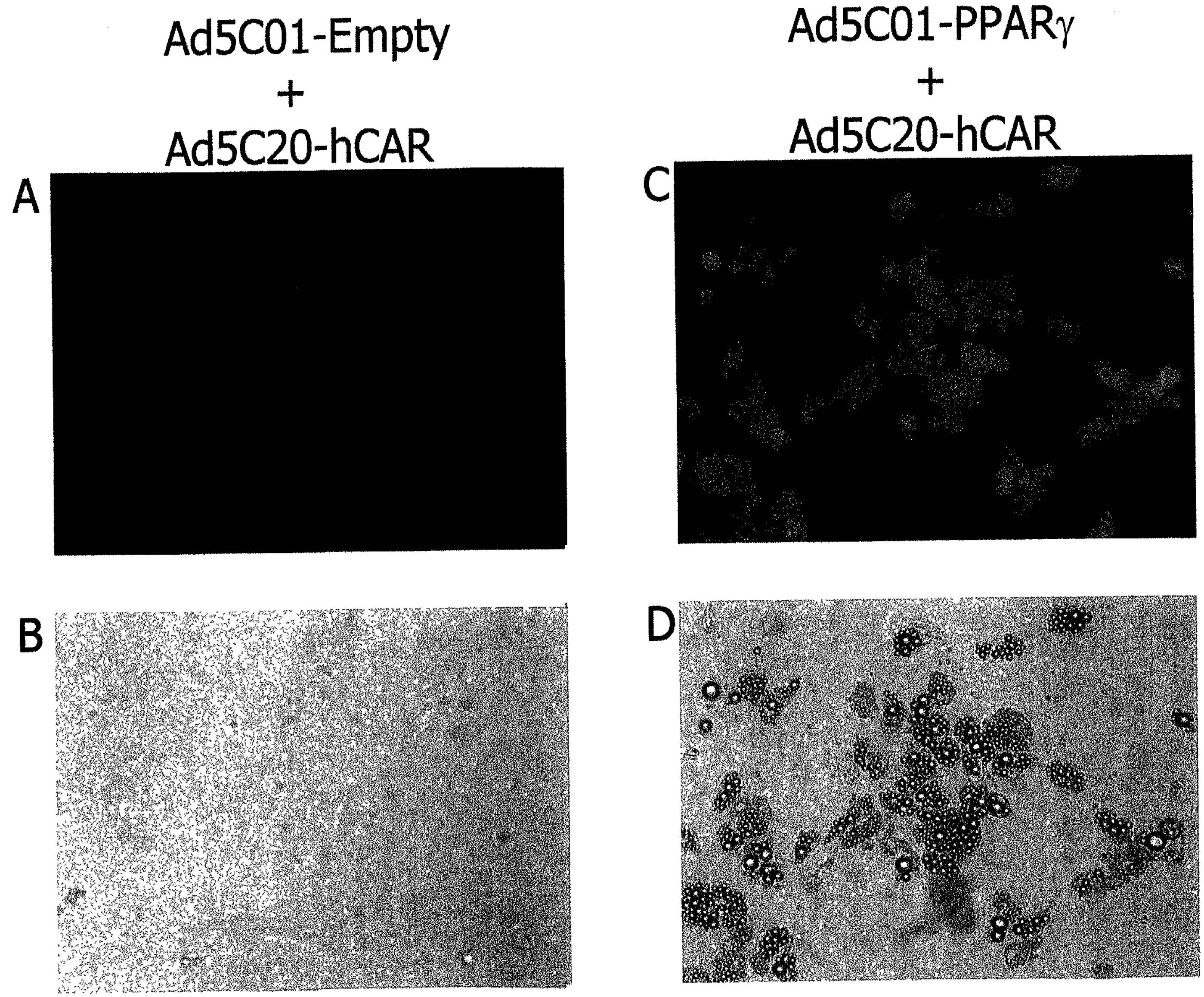


Figure 48



Adipocyte differentiation
Primary human mesenchymal stem cells

Figure 49

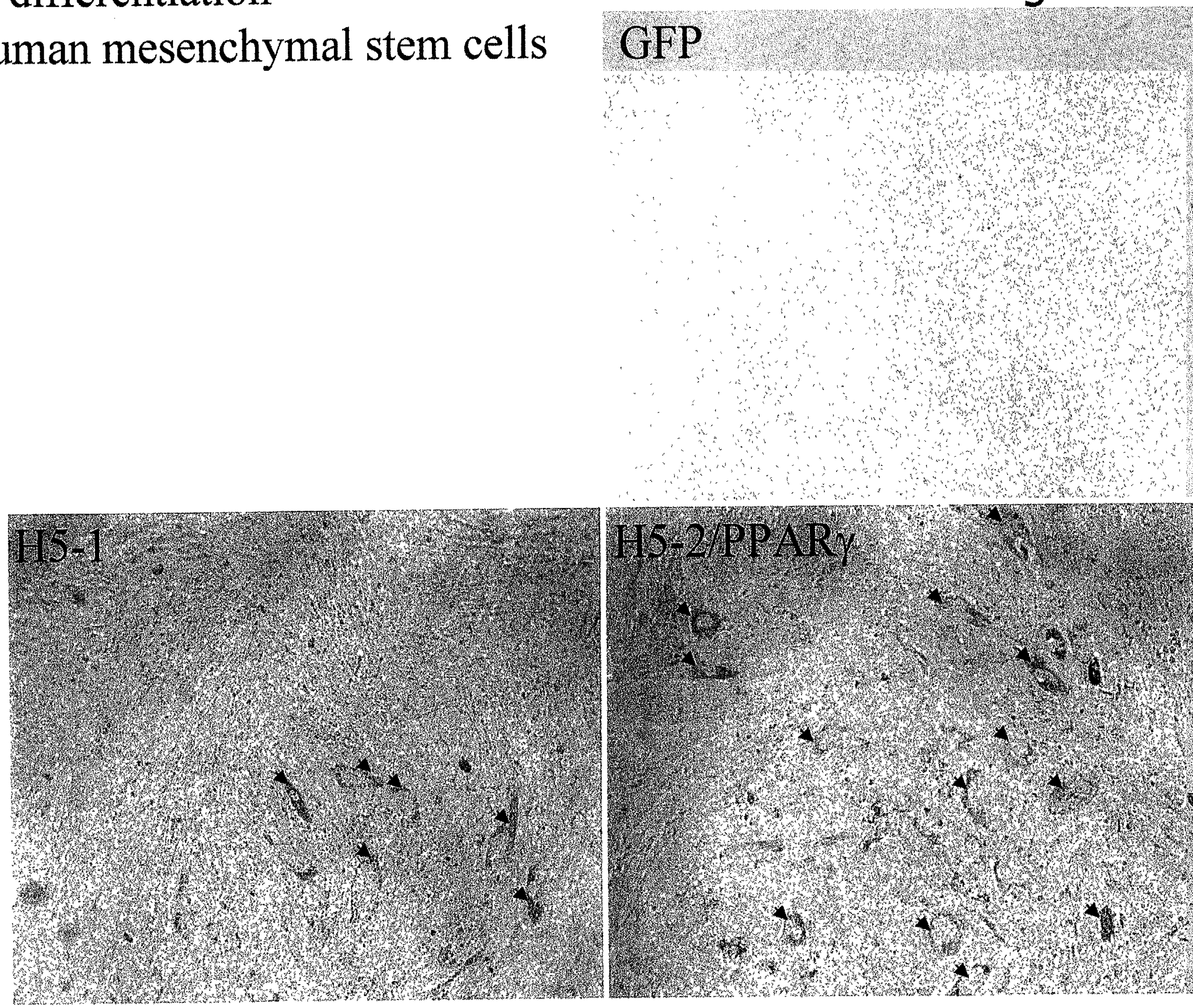


Figure 50

Adipocyte differentiation
Mouse mesenchymal stem cell line
C3H10T1/2

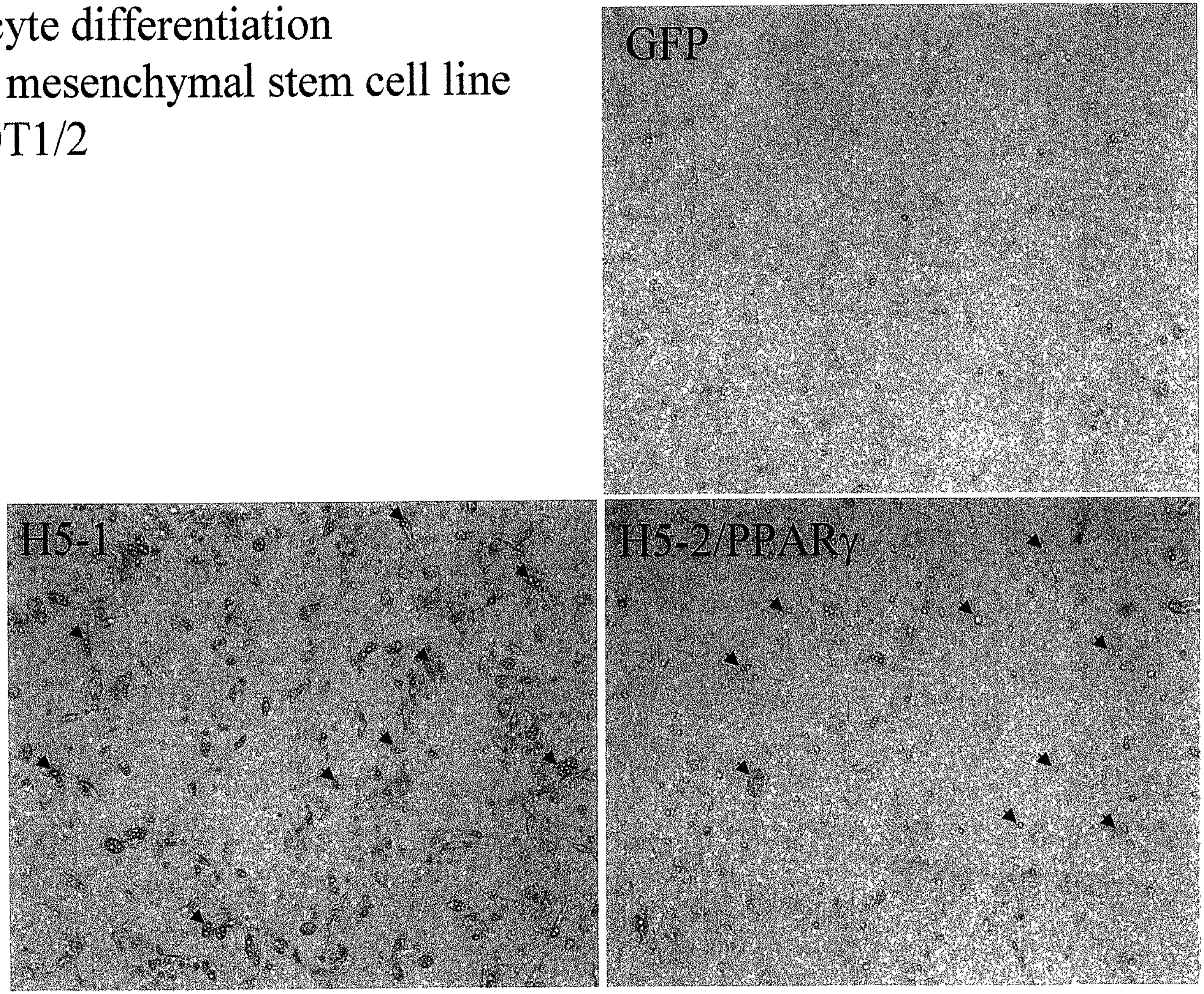
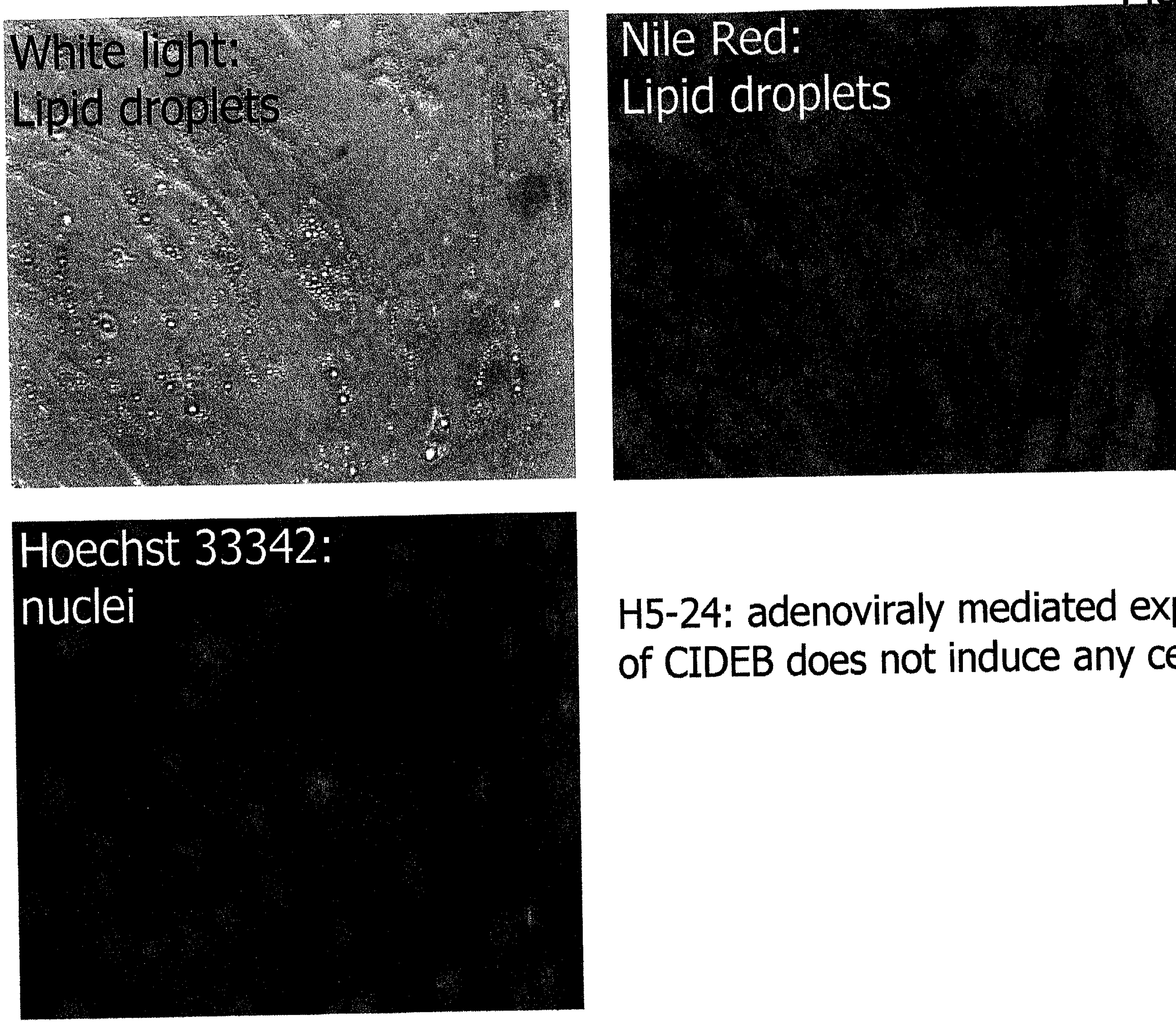


Figure 51



H5-24: adenoviraly mediated expression of CIDEB does not induce any cell death

FIGURE 52

H5-1 DNA sequence (SEQ ID NO:14)

```
   1 GCCCACGCGT CCGGTTTTCT ACTTTGCCAC AGATTATCTT GTACAGCCTT TTATGGACCA
  61 ATTAGCATTC CATCAATTTT ATATCTAGCA TATTTGCGGT TAGAATCCCA TGGATGTTTC
 121 TTCTTTGACT ATAACAAAAT CTGGGGAGGA CAAAGGTGAT TTTCCTGTGT CCACATCTAA
 181 CAAAGTCAAG ATTCCCGGCT GGACTTTTGC AGCTTCCTTC CAAGTCTTCC TGACCACCTT
 241 GCACTATTGG ACTTTGGAAG GAGGTGCCTA TAGAAAACGA TTTTGAACAT ACTTCATCGC
 301 AGTGGACTGT GTCCCTCGGT GCAGAAACTA CCAGATTTGA GGGACGAGGT CAAGGAGATA
 361 TGATAGGCCC GGAAGTTGCT GTGCCCCATC AGCAGCTTGA CGCGTGGTCA CAGGACGATT
 421 TCACTGACAC TGCGAACTCT CAGGACTACC GTTACCAAGA GGTTAGGTGA AGTGGTTTAA
 481 ACCAAACGGA ACTCTTCATC TTAAACTACA CGTTGAAAAT CAACCCAATA ATTCTGTATT
 541 AACTGAATTC TGAACCTTTC AGGAGGTACT GTGAGGAAGA GCAGGCACCA GCAGCAGAAT
 601 GGGGAATGGA GAGGTGGGCA GGGGTTCCAG CTTCCCTTTG ATTTTTTGCT GCAGACTCAT
 661 CCTTTTTAAA TGAGACTTGT TTTCCCCTCT CTTTGAGTCA AGTCAAATAT GTAGATTGCC
 721 TTTGGCAATT CTTCTTCTCA AGCACTGACA CTCATTACCG TCTGTGATTG CCATTTCTTC
 781 CCAAGGCCAG TCTGAACCTG AGGTTGCTTT ATCCTAAAAG TTTTAACCTC AGGTTCCAAA
 841 TTCAGTAAAT TTTGGAAACA GTACAGCTAT TTCTCATCAA TTCTCTATCA TGTTGAAGTC
 901 AAATTTGGAT TTTCCACCAA ATTCTGAATT TGTAGACATA CTTGTACGCT CACTTGCCCC
 961 AGATGCCTCC TCTGTCCTCA TTCTTCTCTC CCACACAAGC AGTCTTTTTC TACAGCCAGT
1021 AAGGCAGCTC TGTCGTGGTA GCAGATGGTC CCATTATTCT AGGGTCTTAC TCTTTGTATG
1081 ATGAAAAGAA TGTGTTATGA ATCGGTGCTG TCAGCCCTGC TGTCAGACCT TCTTCCACAG
1141 CAAATGAGAT GTATGCCCAA AGACGGTAGA ATTAAAGAAG AGTAAAATGG CTGTTGAAGC
1201 AAAAAAAAAA AAAAA
```

FIGURE 53

H5-24 DNA sequence (SEQ ID NO:16)

1 **GTCGACCCAC GCGTCCG**CGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61 CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCACCC GCGCCCCGTG CCGCCCGGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCG
181 AAAGGAAGCA CGAAAGCGGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCGGGC CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCCAGCGG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCCTGG
661 GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGGAC ACAGAAAAAA AGTGGGGAAG
721 CTGGGGGACC CCTACAAGGA TCCTTGGCAG GAAAGCAGGG ATTGTGTTCA TTTGAGGGTT
781 TCACTGTCAG TGAGAGTCTC AGCTTCCATG CAACTGTCCA TCACGGCTGC AACTGAAATC
841 AGAGCTGGGA CACAGCGCAC CAGAAGCTAA AGTCTTGATG CCATCAAAGG ACATCCCTGC
901 CCCATTCACA TCTCTGTCAC GTCCACTAAT CGGCAAAAGG AGAAAAGTGA GAGAAGATGA
961 CCTAAGTGTG ACTGCAGCAG GCAGCTCTGG AAAATGAAGC CAGAGCAGTG AGCCAGCCCC
1021 TCCTCCGACC AAGGAGGAAG GAAAGAGCAG CCCCAGCACA GGAGAGAACC ACCCAGCCCA
1081 GAAGTTCCAG GGAAGGAACT CTCCGGTCCA CCATGGAGTA CCTCTCAGCT CTGAACCCCA
1141 GTGACTTACT CAGGTCAGTA TCTAATATAA GCTCGGAGTT TGGACGGAGG GTCTGGACCT
1201 CAGCTCCACC ACCCCAGCGA CCTTTCCGTG TCTGTGATCA CAAGCGGACC ATCCGGAAAG
1261 GCCTGACAGC TGCCACCCGC CAGGAGCTGC TAGCCAAAGC ATTGGAGACC CTACTGCTGA
1321 ATGGAGTGCT AACCCTGGTG CTAGAGGAGG ATGGAACTGC AGTGGACAGT GAGGACTTCT
1381 TCCAGCTGCT GGAGGATGAC ACGTGCCTGA TGGTGTTGCA GTCTGGTCAG AGCTGGAGCC
1441 CTACAAGGAG TGGAGTGCTG TCATATGGCC TGGGACGGGA GAGGCCCAAG CACAGCAAGG
1501 ACATCGCCCG ATTCACCTTT GACGTGTACA AGCAAAACCC TCGAGACCTC TTTGGCAGCC
1561 TGAATGTCAA AGCCACATTC TACGGGCTCT ACTCTATGAG TTGTGACTTT CAAGGACTTG
1621 GCCCAAAGAA AGTACTCAGG GAGCTCCTTC GTTGGACCTC CACACTGCTG CAAGGCCTGG
1681 GCCATATGTT GCTGGGAATT TCCTCCACCC TTCGTCATGC AGTGGAGGGG GCTGAGCAGT
1741 GGCAGCAGAA GGGCCGCCTC CATTCCTACT AAGGGGCTCT GAGCTTCTGC CCCCAGAATC
1801 ATTCCAACCG ACCCACTGCA AAGACTATGA CAGCATCAAA TTTCAGGACC TGCAGACAGT
1861 ACAGGCTAGA TAACCCACCC AATTTCCCCA CTGTCCTCTG ATCCCCTCGT GACAGAACCT
1921 TTCAGCATAA CGCCTCACAT CCCAAGTCTA TACCCTTACC TGAAGAATGC TGTTCTTTCC
1981 TAGCCACCTT TCTAGCCTCC CACTTGCCCT GAAAGGCCAA GATCAAGATG TCCCCCAGGC
2041 ATCTTGATCC CAGCCTGACT GCTGCTACAT CTAATCCCCT ACCAATGCCT CCTGTCCCTA
2101 AACTCCCCAG CATACTGATG ACAGCCCTCT CTGACTTTAC CTTGAGATCT GTCTTCATAC
2161 CCTTCCCCTC AAACTAACAA AAACATTTCC AATAAAAATA TCAAATATTT AAAAAAAAAA
2221 AAAAAAA**GGG** **CGGCCGC**

FIGURE 54

H5-24 ORF4 Amino Acid sequence (SEQ ID NO:15)

```
  1 MEYLSALNPS DLLRSVSNIS SEFGRRVWTS APPPQRPFRV CDHKRTIRKG
 51 LTAATRQELL AKALETLLLN GVLTLVLEED GTAVDSEDFF QLLEDDTCLM
101 VLQSGQSWSP TRSGVLSYGL GRERPKHSKD IARFTFDVYK QNPRDLFGSL
151 NVKATFYGLY SMSCDFQGLG PKKVLRELLR WTSTLLQGLG HMLLGISSTL
201 RHAVEGAEQW QQKGRLHSY 219
```

FIGURE 55

Region 1 of H5-24 Complementary to BLTR2 DNA sequence (SEQ ID NO:17)

```
                       18 CGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
 61 CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCACCC GCGCCCCGTG CCGCCCGGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCG
181 AAAGGAAGCA CGAAAGCGGT 200
```

FIGURE 56

Region 2 of H5-24 Complementary to BLTR2 DNA sequence (SEQ ID NO:18)

```
                    198 GGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCGGGC CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCCAGCGG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCCTGG
661 GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGG 698
```

FIGURE 57

Alignment of SEQ ID NO:17 with DNA sequence complementary to BLTR2 sequence. SEQ ID NO:17 is 100% identical to antisense BLTR2 DNA.

```
SEQ ID NO:17   18 cgcctgcagaaggttgactgcgtggtagggggcccagagcaagccgaaggcaagcacgat 77
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2455 cgcctgcagaaggttgactgcgtggtagggggcccagagcaagccgaaggcaagcacgat 2396
BLTR2

SEQ ID NO:17   78 ggcgctcaccagccggcccacccgcgccccgtgccgcccggagccccagcgggcgccccg 137
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2395 ggcgctcaccagccggcccacccgcgccccgtgccgcccggagccccagcgggcgccccg 2336
BLTR2

SEQ ID NO:17  138 cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaaggaagcacgaaagc 197
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2335 cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaaggaagcacgaaagc 2276
BLTR2

SEQ ID NO:17  198 ggt 200
                  |||
Antisense    2275 ggt 2273
BLTR2
```

FIGURE 58

Alignment of SEQ ID NO:18 with DNA sequence complementary to BLTR2 sequence.

SEQ ID NO:18 is 100% identical to antisense BLTR2 DNA.

```
SEQ ID NO:18 198  ggtggcggtagacggcggccgggacggcgagcaacagggcggccagccagaccgccagca 257
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2195 ggtggcggtagacggcggccgggacggcgagcaacagggcggccagccagaccgccagca 2136
BLTR2

SEQ ID NO:18 258  gcaggcggcgggccagggccgggctgcgcagccgaggcgccaggaaggggcgggtgactg 317
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2135 gcaggcggcgggccagggccgggctgcgcagccgaggcgccaggaaggggcgggtgactg 2076
BLTR2

SEQ ID NO:18 318  cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgtacatgctgagcg 377
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2075 cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgtacatgctgagcg 2016
BLTR2

SEQ ID NO:18 378  cgcacacgtagtacaccgccttgcagcccgcctggcccagcggccaggcctgccgggtca 437
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    2015 cgcacacgtagtacaccgccttgcagcccgcctggcccagcggccaggcctgccgggtca 1956
BLTR2

SEQ ID NO:18 438  ggaaggccacaaagagcggcgtgagcagcagcaccgcgccgtcggccagcgccaggtgca 497
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    1955 ggaaggccacaaagagcggcgtgagcagcagcaccgcgccgtcggccagcgccaggtgca 1896
BLTR2

SEQ ID NO:18 498  gcacaagcgtggccgccagcggtcgcccccgtgcaggccgccagcccgccaagctccaca 557
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    1895 gcacaagcgtggccgccagcggtcgcccccgtgcaggccgccagcccgccaagctccaca 1836
BLTR2

SEQ ID NO:18 558  ccacgaagccgttgccaggcagccccagcagcgccgccagcagcaggaaggctgtgcctg 617
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    1835 ccacgaagccgttgccaggcagccccagcagcgccgccagcagcaggaaggctgtgcctg 1776
BLTR2

SEQ ID NO:18 618  tggcccgcgaagtcttccagctcagcagtgtctcgttccctgggggacggtagcagaccg 677
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Antisense    1775 tggcccgcgaagtcttccagctcagcagtgtctcgttccctgggggacggtagcagaccg 1716
BLTR2

SEQ ID NO:18 678  acatccttctgggcctacagg 698
                  |||||||||||||||||||||
Antisense    1715 acatccttctgggcctacagg 1695
BLTR2
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